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Ameliorative Effects of Vitamin-E and Selenium Supplementation on Arsenic-Induced Toxicity in Japanese Quails (*Coturnix Japonica*)

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Abstract

THIS study explored the ameliorative effect of vitamin E (Vit. E) and selenium (Se) against arsenic (As)- induced toxicity in Japanese quails (*Coturnix Japonica*). Three hundred seven-day-old quails were divided into six groups (n=10/replicate, 5 replicates/group): T1 basal diet (BD; negative control), T2 BD + 20 mg/kg As (positive control), T3 BD + 0.3 mg/kg Se + 250 mg/kg Vit E, T4 BD + 20 mg/kg As + 250 mg/kg Vit. E, T5 BD + 20 mg/kg As + 0.3 mg/kg Se, and T6 BD + 20 mg/kg As + 0.3 mg/kg Se + 250 mg/kg Vit. E). At 35 days, six birds per group were slaughtered for different histological and serological analysis. The supplementation of Vit. E and Se significantly improved feed intake in T3, T4, and T6 groups than the control groups, while body weight gains and feed conversion ratio (FCR) were superior in T3 and T4 groups. The serological data show improvements in serum glucose and triglycerides levels in T3-T6 than in T1 and T2 groups. The levels of uric acid and creatinine were also improved in T3 and T6 groups. However, malondialdehyde (MDA) levels were reduced, and catalase (CAT) levels were increased in T4-T6 groups than in T2 group. Histomorphometrically, the muscle fiber and fascicle diameter, intestinal villus height (VH), crypt depth (CD), and VH:CD ratio were improved in T3–T6 than in T2 groups. In conclusion, these findings suggested that dietary Vit. E and Se may alleviate As-induced oxidative stress and cellular damage in quail birds.

Keywords: Arsenic toxicity; quail birds; oxidative stress; vitamin E; selenium.

Introduction

Despite being a major health risk in Asia, heavy metals like arsenic are among the most sensitive environmental issues in Pakistan. In developing nations, it causes a major public health issue. Arsenic can spread widely throughout the kingdoms of plants and animals by getting into the food chain. Its a common non-essential trace element found in nature, arsenic is shiny and greyish compound[1,2]. Both its organic and inorganic forms are accessible all over the world. The most hazardous forms of arsenic are found in inorganic forms (FAO, 1983). Arsenic, one of the most powerful poisonous metalloids in the environment, is included as the first compound on the Substance Priority List 2013 [4] by the Agency for poisonous Substances and Disease Registry (ATSDR). By consuming tainted food and drinking water, millions of people worldwide are being exposed to inorganic arsenic [5]. Heavy metals have the ability to build up in an organism's tissues and organs, which can cause long-term toxicity in animals and birds [6]. Thus, quail birds may become poisonous due to high or excessive quantities of arsenic in food and water, as well as from consuming tainted feed and water for an extended period of time.

Selenium has been identified as an antioxidant and is a necessary trace element for animals [7]. Pretreatment with selenium protects the frontal cortex, striatum, and hippocampal regions against oxidative damage caused by restraint stress [8]. Due to the fact that greater dosages of selenium not only

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injure kidneys but also hinder reproduction and increase stress in birds [9]. Reactive oxygen species (ROS) are produced in greater quantities under stress, which damages lipids and proteins [10]. Because stress reduces feed intake, body weight, and body weight gain in broilers, it has a detrimental effect on growth performance [11]. It exacerbates inflammation and the burden of microbial illnesses in the gut, resulting in harmful outcomes like food indigestibility, diarrhea, and electrolyte loss [12].

Furthermore, it decreases the growth of immune organs like the bursa of Fabricius in broilers, reduces the intestinal absorptive surface area and decreases organ weights [13]. Selenium's ability to prevent liver damage caused by aluminum toxicity was previously demonstrated [14]. Selenium also have protective benefits against oxidative damage in trophoblast cells and prevents against testicular toxicity in cocks caused by cadmium as reported by Watson., 2012 [15]. Alpha tocopherol is the most biologically active of a group of lipo-soluble chemicals generated from tocol and tocotrienol that are collectively referred to as vitamin E (Vit E). The ability of vitamin E's molecular structure to fix ROS types O_2^-, O_2^-, and OH^- is what gives it its "biological antioxidant" property [16].

In cell membranes, vitamin E serves as the body's first line of defense against free radicals and oxidative stress [18]. Because of its anti-inflammatory genetic expression in broilers, it enhances gut health [19]. It preserves the integrity of the intestinal basement membrane and raises the height of the intestinal villus [20]. By raising the mucosal antibody titer in stressed-out hens, vitamin E enhances the intestinal immune response. By making the immune organs heavier, it acts as an immunomodulator because the relative weight of these organs improves the broiler's immunological response [21, 19]. Together with other antioxidants, vitamin E helps to protect the cells from damage.

Vitamin E is a non-enzymatic antioxidant, whereas selenium is an enzymatic antioxidant [22]. By lowering malondialdehyde (MDA), they work together to fight free radicals that are created during stressful situations [23]. By boosting body weight and favorably influencing growth hormone expression in birds, they also work in concert to enhance growth performance [24]. In a study it is found that in broilers and quail birds, combined use of vitamin E and selenium increases the weight of the spleen and bursa [25].

To the best of our knowledge, the scanty information about arsenic toxicity in quail birds is available; therefore, the present study was designed and executed with the objectives to know the pathologic and oxidative alterations induced by arsenic in quail birds and whether vitamin E and selenium ameliorate these alterations or not.

Material and Methods

Ethical statement

This study, and all associated procedures, were approved and performed according to rules and regulations of the Ethical Review Committee at the Department of Veterinary Anatomy and Histology, University of Veterinary and Animal Sciences, Lahore, Pakistan (Ethical Approval No. DR/202; Dated:05/05/2023).

Materials

Experimental study design and procedure

This study was carried out at the Avian Research and Training (ART) Centre, University of Veterinary and Animal Sciences, Lahore. A total of 300 (n = 300) seven-day-old mixed male and female Japanese quails were used in this experiment and assigned to six groups of five replicates per each of those groups at 10 birds in each cage. The supplementation of the experimental groups were determined as follows: the birds in the negative control group (T1) were given a basal diet only; the positive control group (T2) was fed BD + As 20 mg/kg; T3 was supplemented with Se 0.3 mg/kg + Vit E 250 mg/kg + BD; T4 was supplemented with As 20 mg/kg + Vit E 250 mg/kg; T5 was supplemented with As 20 mg/kg + Se 0.3 mg/kg; and T6 was supplemented with As 20 mg/kg + Se 0.3 mg/kg + Vit E 250 mg/kg + BD. The quantities of selenium and vitamin E were given according to the study [26] from the seven day age of chicks until the end of the experiment. The quails were kept in a six-tier battery cage system. The birds were fed ad libitum and had access to clean water through a nipple drinker system. A temperature of 35℃ ± 5℃, relative humidity at 65 ± 5%, was maintained. Drop trays were placed to obtain collected manure. The use of selenium and vitamin E started from the first day of the experiment. The time of the trial lasted for 35 days.

Sample collection

On the 35th day of the experiment, two randomly selected quails from each replicate were slaughtered by the Zabiha (cutting jugular and carotid arteries, leaving the spinal cord intact) method using a sharp blade. The muscle tissue from the chest area pectorals was removed manually by using the scalpel blade, and a segment of intestine from the duodenum, jejunum, and ilium was collected for histomorphometry of the intestine. A 2 cm piece of each of the small intestine was collected and washed with cold normal saline after the intestine was opened longitudinally. These small intestine pieces were placed in 10% neutral buffered formaldehyde. The same process was done to each lymphoid organ which included as spleen and bursa. The immune organs were collected at the end of the experiment and weighed on electrical balance for histomorphometry.

Body weight gain and FCR

To assess growth performance, all of the quails weighed in by using an electrical balance on arrival and then on days 7, 14, 21, and 28. During those time frames, weekly body weight gain was calculated for the entire replicate using the following formula:

Body weight gain (g) = final body weight (g) - initial body weight (g)

FCR was calculated using the following formula every week:

Feed conversion ratio = feed intake (g)/body weight gain (g)

Biochemical analysis and antioxidants

Glucose was measured using the glucose GOD-POD enzymatic colorimetric kit Ref. 101-0238 from CHRONOLAB (Spain). To measure triglycerides, the triglycerides GPO-POD colorimetric method and liquid reagent Ref. 101-0579 from CHRONOLAB (Spain) were used. Creatinine was measured using the creatine kinase–mb kit Ref. 101-0375 from CHRONOLAB (Spain). Uric acid was measured using the uric acid colorimetric method (POD) and liquid reagent kit Ref. 101-0528 from CHRONOLAB (Spain).

Serum cortisol, cholesterol, catalase, and MDA levels were evaluated. Blood was drawn, and serum was obtained by centrifuging the blood at 1500 rpm for 10 minutes. Serum was kept at -40°C for further analysis. Serum cortisol was evaluated using a commercially available cortisol ELISA kit (Calbiotech U.S.A., REF CO368S, LOT COS5342), while serum total cholesterol was evaluated using a commercially available kit (Human GmbH, Germany, REF 10017, LOT 16008). The MDA (malondialdehyde) level was ascertained from serum using the previously described method [27]. while the catalase activity was evaluated using the method of Hadwan and Abed [28].

Processing of muscle tissues

To undertake histology of pectoral muscle, muscle tissue was collected and preserved in neutral buffered formalin after slaughter and was then dehydrated by passing it through a series of alcohols of increasing concentration: 70%, 80%, 90%, and 100%. The tissue samples were then cleared in xylene. The tissues were then infiltrated for 6 h in molten paraffin at 58°. Using metal and plastic disposable molds, tissue blocks were produced. Tissue sections of 4-5 µm thickness were obtained from the tissue blocks using microtomy. The sections were placed in a water bath containing gelatin powder at 45-50°C, and the sections were then mounted on glass microscope slides in the water bath and stained with haematoxylin and eosin stain (H&E).

Muscle fiber and fascicle diameter

For fascicle diameter, the images of slides were captured at 4X from different areas of the slide. In the determination of muscle fiber diameter (µm), the microscopic slides of the muscle cross section stained with H & E were viewed at 10X on a bright field microscope. Muscle fiber diameter was calculated by the histomorphometry program (Progress Capture Pro 2.7.7, Labomed USA). The diameters (µm) of five muscle fibers from three fascicles were measured, and the average of muscle fiber diameters was calculated. Likewise, fascicle diameters were measured. The cross-sectional area of the muscle fiber was measured from the muscle fiber diameter.

Muscle fiber count

For the total number of muscle fiber count, slides that were stained with H&E, it was observed at 4X and calculated by software (Progress Capture Pro 2.7.7, Labomed USA). The number of muscle fibers on three randomly selected fascicles was counted, and the muscle fibers per unit area was evaluated.

pH and water holding capacity

The capacity to hold water was determined using the method described by El-Refaiy [29]. For this, a 1-g cube of meat was sandwiched between two glass plates and loaded with 8-10 kg of weight for 5 min. Observations were made as to the initial and final weights, and the amount of water lost as meat weight was recorded. The pH of muscle also is an indicator of rigor mortis development. The accumulation of lactic acid that occurs once muscle is dead causes the pH of muscle to drop. Mohod and Dhote reported that stress prior to slaughter elevates the glycolytic rate, resulting in pale, soft, exudative meat [30].

The most important meat quality parameter for assessing the sensory quality of meat is pH [30]. It has been documented that pH changes in muscle have an impact on meat quality [31] and meat quality parameters include meat color, muscle texture and water-holding capacity. To determine muscle sample pH, a conventional probe electrode was inserted into muscle tissue one cm deep into the muscle. Color readings were recorded using a calibrated Minolta meter (Konica Minolta CR-140, Japan) at room temperature.

Intestinal morphometric evaluation

In the small intestine, morphometric parameters such as crypt depth (CD), lamina propria thickness (LPT), muscularis mucosa thickness (MMT), muscularis externa thickness (MET), villus length (VL), width (VW), surface area (VSA), and crypt depth (CD) were measured for histomorphometry [32]. All five (5) well-organized villi were taken into consideration for measuring VL, VW, and CD for each intestinal slide cross section. The distance

measured from the villus tip to the villus crypt junction was called the villus length (µm). VH:CD was calculated using the crypt depth and villus height data. VH:CD was calculated using the crypt depth and villus height measurements. Three (3) locations were used to measure the villus width (VW), including the villus tip, the middle and base of the villus. Average of these three values were used as width of villus. Villus surface area (VSA) (µm²) was determined by using formula: (2 × 40 Experiment 2 3.14) × (VW ÷ 2) × (VL). Lamina propria thickness (LPT) and muscularis externa were measured [33].

Morphometric parameters of immune organs

Under three microscopic fields of tissue samples at 4X, the number, length, width, and area of five lymphatic nodules (LN) were determined, and average values were obtained. To calculate the area of lymphatic nodules (LNA), the following formula was utilized. LNA: (NW) x(NL), where NL is the nodular length or height and NW is the nodular width. Using a light microscope (4X), the total number, length, width, and area of lymph follicles in the bursa of Fabricus were determined in three microscopic areas of each tissue sample. Three sections' mean values were calculated. The following formula was used to determine the lymph follicle's (LFA) area. LFA: (FW ×FL) where FW follicular width FW and FL is length follicle [33]. In thymus histomorphometry, two lines that crossed at a right angle in the middle of the medulla divided the lobules into four sections. The representative lines were used to measure the cortical width (um), and the average mean was displayed as the cortex thickness. The medulla's length and width (L × W) were used to calculate its area. Three well-organized lobules per section were used to calculate the cortex to medulla ratio. Three sections per bird were used to determine the mean values [34, 35].

Statistical analysis

Data was analyzed using one-way ANOVA in SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). Tukey's post hoc test employed to compare treatment means. Results are expressed as mean ± standard error of the mean (SEM). Level of significance was set at $P < 0.05$.

Results

Weight gain and FCR

Physical parameters like body weight, FCR and feed consumption decreased significantly ($P<0.05$) in arsenic-supplemented groups of Japanese quails. In the arsenic-supplemented group, there was increased FCR compared to the control group. While groups supplemented with Vit E, selenium and a combination of Vit E and selenium, separated and along with arsenic, exhibited significantly ($P<0.05$) improved FCR (Table 2). Effects of vitamin E and selenium supplementation on growth performance, feed conversion ratio and average daily gain of Japanese quails were recorded during the 35th day of the experiment.

Antioxidant enzymes

The levels of CAT for Japanese quails administered with arsenic, vitamin E, selenium alone and in combination have been presented in Table 3. A significantly higher CAT level was observed in the control group, and $P<0.05$ was recorded in the control group. In its comparison the lowest CAT value was observed in the arsenic plus selenium-supplemented group, and $P<0.05$ was observed in this group. While the group treated with vitamin E and selenium showed no significant results ($P > 0.05$). CAT values were compared with the control group, and the selenium-supplemented group showed a significantly lower value of CAT on the 35th day of the experiment.

Malondialdehyde (MDA) concentration for Japanese quails administered with arsenic, vitamin E, selenium alone and in combination has been presented in Table. 2. On the 35th day of the experiment, the highest value of MDA was recorded in the control positive group and the vitamin E-supplemented group. A significantly higher value of MDA was recorded in the vitamin E supplement where $P < 0.05$. However, the lowest value of MDA was observed in the control group with a significant difference of $P<0.05$. Higher MDA values were recorded in the vit E, selenium and arsenic-supplemented group (Table 4).

Histomorphometric analysis of small intestine

The effect of vitamin E and selenium separately and in combination on arsenic toxicity-induced Japanese quails was observed. Results showed increased villus height (VH) and reduced crypt depth (CD) in the duodenum, jejunum and ileum, considering $P<0.05$. While results revealed an increased VH:CD ratio in the vitamin E and selenium-supplemented group ($P<0.05$). Effect of vitamin E and selenium supplementation on intestinal morphology of quails intoxicated with arsenic, means in the row bearing (a, b, c, d) differ significantly at $P > 0.05$ and are shown in Table 3 and Table 5.

Histology of muscle samples

pH and water holding capacity of meat

The water holding capacity was measured according to the method described by El-Refaiy [29]. For which 1 g meat cube pressed between two glass plates and applied 8–10 kg weight for 5 minutes. The initial and final weight readings before and after the procedure were recorded, and the difference in their weight represented the water loss.

The development of rigour mortis is also indicated by muscle pH. Accumulation of lactic acid

within the muscles results in the drop of pH of the muscle. As reported by Mohod and Dhote [30, 36] Stress prior to slaughtering can lead to increased glycolysis, due to which pale, soft, exudative meat is produced. However, in the current study, no significant change in pH was seen among the supplemented and control groups.

Histology of immune organs

Light microscopic observations revealed that, in the arsenic-administered group, cortical thickness of bursal tissue was reduced while the medullary region was having severe lymphocytic necrosis and a more enhanced area. Arsenic also led to the edema of the medullary region and fibrosis of the interstitium in the bursal tissue of quail birds. Supplementation of vitamin E and selenium significantly inhibited the disruption induced due to arsenic administration. After the addition of vitamin E and selenium, the severity of lesions was reduced in the bursa of Fabricius. A decrease in the follicular size is attributed to deteriorative changes observed in the bursa of quail birds. A number of studies suggested that antioxidants like vitamin E alleviate toxic effects. The tissue-protective effect of vitamin E is due to its antioxidant activity [29].

In accordance with our study, Hussein et al. described lesions of the bursa, depletion of the central follicular portion and degenerative changes in the epithelial cells which are lining the mucosal layer as due to arsenic toxicity. Results of this study revealed that vitamin E and selenium supplementation protects the bursa of Fabricius against toxicity induced by arsenic in Japanese quails. Thus, it can be concluded on the basis of results that additives have a significant effect in decreasing the effects of toxicity in the bursa of quail birds.

Gross histological parameters of organs

Gross and microscopic lesions recorded in Japanese quails administered vitamin E and selenium alone and in combination after exposure to arsenic toxicity are shown in Table 6. In gross observation, the liver was of normal size, shape and consistency in treated groups. While microscopically advanced fatty changes in hepatocytes with pyknosis and nuclear congestion were observed, which are shown in Table 6. In hepatic lobules, separation of cells from the basement membrane and infiltration of mononuclear cells were observed, which are shown by the +, ++ & +++ in Table 6, and absence of such change is marked with -. While in some treated groups, cell detachment from the basement membrane, in large amounts, fatty changes and more extended sinusoidal changes were observed in some treated groups. In Japanese quails treated with heavy metals, nuclear degeneration and karyolysis with the use of arsenic were observed. While the control group showed no microscopic lesions in hepatic lobules hepatocytes.

Discussion

The study evaluated how dietary vitamin E and selenium could protect Japanese quails from arsenic-induced damage. According to the findings, adding these antioxidants to the diet considerably enhanced feed intake, body weight gain, feed conversion ratio (FCR), hematobiochemical indicators, intestinal and muscle histomorphological structures, and immune organ development. When compared to control groups, birds supplemented with vitamin E and selenium (Se) performed better in terms of body weight gain and FCR. According to earlier observations, birds treated with arsenic showed a considerable decrease in body weight and feed intake [37, 38]. [39] It has been reported that arsenic's inhibitory effect on a particular region of the brain's hypothalamus, which regulates feed intake, eventually results in limited feed intake and decrease in body weight gain. Furthermore, metabolic deregulation brought on by the harmful effects of heavy metals on the liver in birds may potentially be the cause of this decreased feed intake and body weight [40].

Our study showed improved tendency in glucose and triglyceride levels by the supplementation of vitamin E and Selenium as compared to the control groups. Analysis of our data suggested significant decrease in the level of urea and creatinine in supplemented groups as compared to the control groups. Our results are similar with the findings of [49,50] who suggested increase in level of urea and creatinine in ducks, goats and rats due to heavy metals toxicity. This increase in the level of urea and creatinine is due to Lipid peroxidation which increases due to the generation of ROS. Sedimentation of lipid droplets in the glomerular endothelium, damages the membrane components even necrosis can occurs which ultimately effects the glomerular filtration rate (GFR) thus a elevation in the level of urea and creatinine occurs [51].

Antioxidants are chemicals that postpone or stop cellular damage by reducing or preventing free radical reactions. Cells are protected by antioxidants from the damaging effects of free radicals. ROS, which are produced by heavy metals, harm lipids and proteins [41, 46]. Oxidative stress is the process by which ROS triggers antioxidant signaling between cells, increasing antioxidative capacity, while cells regulate ROS levels through their antioxidative defense systems [42, 43]. Most cell types don't really care about catalase (CAT) under normal circumstances, but when oxidative stress is present, it is the most adaptable antioxidant enzyme and is crucial for cellular defense against oxidative damage [44, 45]. Malondialdehyde (MDA) is one of the several end products that are produced during the

breakdown of lipid hydroperoxides. By lowering the levels of antioxidant enzymes (CAT, SOD, and GPx) and increasing lipid peroxidation, heavy metals such as arsenic induce oxidative stress [47]. In Japanese quails, vitamin E functions as an antioxidant by raising CAT levels and decreasing MDA levels. It is a non-enzymatic antioxidant that protects polyunsaturated fatty acids from free radicles that are produced in stress condition [49]. Vitamin E acts as immunomodulatory by decreasing malondialdehyde (MDA) in immunocompromised birds [48, 50]. The results of this study suggested that antioxidant enzymes MDA and CAT showed a significant improvement in the supplemented groups where the value of MDA was decreased and value of CAT was increased in these groups as compared to the control positive group. Our results were similar to the findings of [58] where the value of total oxidant capacity (TOC) and MDA increased significantly in heavy metal administered broiler chicks.

The findings of histomorpometric analysis of pectoral muscle showed a significant improvement in muscle fiber and fascicle diameters of supplemented groups than the control groups. Our results are similar to the [51] who reported the increase in muscle fiber diameter and water holding capacity of pectoral muscle in Japanese quails supplemented with vitamin E and selenium. This is due to better cell integrity and hydration which improves due to the antioxidant properties of the supplements [51].

[52] Li reported that intestinal health improves due to anti-inflammatory action, modulation of antioxidants balance and abundance of immunity related genes in intestine. According to [53] the production of free radicles disrupt the tight junctions of epithelium in small intestine, and during physiological stress corticotrophin releasing factor is a source to release proteases which changes the permeability of intestine. Moreover, the mucosal area and villus height are considered the indicators of intestinal absorptive capacity and VH:CD represents overall functional capacity of intestine [54]. In this study, the absorptive parameters of intestine were adversely affected by the Arsenic toxicity. Because arsenic is found to delay the apoptosis and increase the viability of enterocytes due to its antioxidative role as a results of which it increases villus height [55]. One of the contributing factors to increase intestinal health is down regulation of pathogenic bacteria and up regulation of beneficial bacteria such as Lactobacillus ssp and Faecalibacterium spp and selenium is found to increase the later ones [56]. For the analysis of tissues of small intestine (duodenum, jejunum & ilium), this study observed that the values of villus height (VH), crypt depth (CD), VH :Cd ratio were significantly higher in dietery supplemented groups than the control groups. These findings were similar to the [57] who reported that length of intestinal villi increases in red-legged partridges with dietary inclusion of Selenium and vitamin E.

In this study, the bursal lymphatic follicular length and areas were found greater in supplemented groups than the control groups. This is because of dietary selenium that improves the proliferation of B-lymphocytes and increased lymphatic follicular area [58]. According to [59] the selenium deficiency activates the expressions of IL-6, TNF-α mRNA and toll like receptors TLR signaling pathways which causes the inflammatory injury in bursa of Fabricuis. The results of this study were similar with the outcomes of [60] who reported that the decrease in the length, width and area of bursal follicles occurs in poultry birds exposed with heavy metals toxicity. This study also observed the greater bursal follicular in positive control group than the supplemented groups, which indicated that the size of bursal follicles were smaller in positive control group. Similarly [61] reported that vaculation of epithelial cells in bursal cortex and medulla were markedly reduced by the addition of selenium and vitamin E in feed.

In the present study, the length of splenic nodule was higher in all supplemented groups, and the width of splenic nodule was higher in the groups Se and Se+Vit.E than in the group PC. The possible reason is that the deficiency of selenium causes inflammation and apoptosis, redox imbalance and alters the expression of selenoproteins in the spleen [62] Similarly, the deficiency of alpha tocopherol causes the elevation of MDA level, which suppresses the Nrf2-regulated antioxidative system and induces apoptosis and oxidative damages to the structural integrity of the spleen [63]. These studies suggest that the optimal levels of selenium and vitamin E are necessary to be added in feed to alleviate undesirable impacts of oxidative stress induced by glucocorticoids.

The lymphoid depletion and vacuolation in epithe lial cells in the spleen and bursal medulla and cortex were markedly reduced by the addition of selenium and vitamin E in feed [61]. The kidneys are an organ that is particularly susceptible to arsenic toxicity, as it can have fatal effects on the renal tubular epithelium in chickens in a dose-dependent manner [64]. Due to the constant generation of reactive oxygen species (ROS) and reactive nitrogenous species in these organs, which cause damage to the thiamine nucleotide of DNA strands, early exposure to inorganic arsenic may be the cause of hepatocyte and kidney cell neoplastic transformation [65]. According to [66], quail's respiratory system, which includes the liver, kidney, trachea, and lungs, may be particularly affected by the high toxicity of arsenic. The groups treated with arsenic in this study had both microscopic and gross lesions in the liver, kidney, and lungs. Fatty degenerations, sinusoidal space expansion, cells that were separated from the

basement membrane, and cytoplasmic vacuolar degenerations were all seen in the liver. Under a microscope, the lungs showed congestion, edema, bronchial septa, necrosis, and thickening of the alveolar walls, while the trachea of the arsenic-treated birds had hemorrhages and frothy exudate. However, glomerular shrinkage, necrosis of epithelial cells, and vacuolar degeneration of cytoplasm were noted in the kidney examination. In conclusion, compared to the groups who received arsenic alone or arsenic + selenium supplements, respectively, the administration of vitamin E and selenium produced a partial amelioration. These results showed that vitamin E and selenium co-administration might lessen the harmful effects and oxidative stress caused by arsenic in quail birds.

Conclusion

Arsenic toxicity remains a critical concern in poultry production due to its widespread presence in feed and environmental contamination. The present study demonstrated that excessive arsenic exposure led to oxidative stress, impaired biochemical indices, altered histoarchitecture, and poor performance in Japanese quails (*Coturnix japonica*). Supplementation with vitamin E and selenium, particularly in combination, significantly ameliorated these adverse effects by enhancing antioxidant defenses, improving feed conversion efficiency, and preserving tissue integrity. These findings suggest that dietary antioxidants can be an effective strategy to counteract heavy metal-induced toxicity in avian species. Future studies should focus on elucidating the underlying molecular mechanisms of protection, evaluating the long-term safety and efficacy of such supplementation, and assessing their applicability in commercial poultry farming under varying environmental and management conditions.

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Author contributions

SM, HZ and HR designed the experiment. MZ performed the field and laboratory, analyzed data and drafted the manuscript, by getting inputs from SM and HZ. IA helped in sample collection. SM, HZ and HR reviewed the manuscript.

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Conflicts of interest

The authors have declared no conflict of interest.

TABLE 1. Composition of the Basal diet.

Ingredient	Value (%)	Nutrient Analysis	
Corn	51.56	ME, Kcal/g	2900
Soybean meal, 48% CP	36.73	Crude protein	23
Soybean oil	2.98	Phosphorus	0.4
Methionine	0.15	Calcium	0.9
Calcium carbonate	5.4	Methionine	1.7
Mineral premix	0.25	Lysine	1.3
Trace mineral premix	0.24	Potassium	0.97
Sodium chloride	0.18	Di-calcium phosphate	0.9
Sodium bicarbonate	0.20		

TABLE 2. Effect of Heavy metals toxicity and supplementation of Feed intake, BWG and FCR of 35 days old Japanese Quails.

Parameters	Negative Control	Positive Control	Se+Vit.E	As+Vit. E	As+Se	As+Se+Vit.E
Feed taken (g/day)	$17.24^{ab} \pm 3.07$	$15.5^{b} \pm 1.07$	$17.8^{a} \pm 1.09$	$17.81^{b} \pm 3.03$	$16.95^{a} \pm 2.09$	$17.14^{a} \pm 1.19$
Body Weight gain (g)	$4.11^{b} \pm 1.10$	$3.95^{a} \pm 1.00$	$4.65^{b} \pm 1.36$	$4.45^{b} \pm 1.28$	$4.02^{b} \pm 0.81$	$4.32^{a} \pm 1.32$
FCR	$3.98^{b} \pm 0.98$	$3.67^{a} \pm 1.14$	$4.04^{b} \pm 1.03$	$4.01^{b} \pm 1.28$	$3.94^{a} \pm 0.83$	$3.87^{a} \pm 1.36$

TABLE 3. Effect of Heavy metals toxicity and supplementation of Vit-E and Se on serum biochemical characteristics of 35 days old Japanese Quails

Parameters	Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	P-value
Cholesterol (mg/dL)	88.3^{ab}± 2.05	106.5^{b}± 1.07	85.7 ^{a} ± 2.16	91.17^{b}± 2.98	90.34 ^{b}± 1.04	92.43 ^{a} ± 3.96	0.001
Glucose (mg/dL)	242 ^{b} ± 4.13	221^{ab} ±5.24	261 ^{a} ± 3.39	287 ^{b} ±5.98	245 ^{b} ± 4.02	251 ^{a} ± 5.21	0.000
Triglycerides (mg/dL)	273 ^{ab} ± 5.95	265 ^{b}± 4.47	298 ^{a} ± 5.16	312 ^{b} ± 6.31	279 ^{b} ± 5.04	285 ^{b} ± 5.91	0.002
Uric Acid (mg/dL)	7.46 ^{b} ± 0.96	8.29^{ab}± 0.14	5.7 ^{b} ± 1.03	6.34 ^{a} ± 0.98	6.97 ^{b} ± 0.83	6.2 ^{b} ± 0.36	0.001
Creatinine (mg/dL)	1.86 ^{ab} ± 0.46	1.92^{b}± 0.16	1.88 ^{b} ± 0.85	1.19 ^{a} ± 0.98	0.96 ^{a} ± 0.83	1.14 ^{b} ± 0.74	0.000

TABLE 4. Effect of Heavy metals toxicity and supplementation of Vit-E and Se on histomor- phometric characteristics of CAT and MDA in 35 days old Japanese Quail.

parameters	Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	P-value
CAT Kilo U/L	62.1 ^{ab} ± 3.07	35.23^{b} ± 1.07	64.37^{a}±1.09	39.64^{b}±3.03	34.53±2.09	40.13^{a}± 0.19	0.001
MDA nmol/L	10.26^{ab}± 0.13	13.46 ^{b}± 0.12	9.02^{a} ±0.19	11.62 ^{b}±0.11	12.24 ± 0.12	11.03 ^{a} ±0.10	0.000

TABLE 5. Effect of Heavy metals toxicity and supplementation of Vit-E and Se on histo-mor phometric characteristics of Small intestine in 35 days old Japanese Quails

Intestinal Traits		Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	P-value
Duodenum	VH (µm)	854.8 ^{c} ±0.56	821.3^{d} ± 0.84	985.6^{a}±0.36	856.7^{c}±0.38	834.12 ^{c} ±0.59	862.43^{b}±0.86	0.001
	CD (µm)	79.18 ^{a} ±0.51	89.32^{c} ± 0.64	71^{b} ± 0.84	80^{b} ± 0.73	77^{b} ± 0.56	74^{b} ±0.42	0.000
	VH:CD	10.53^{c} ± 0.19	9.32^{c} ± 0.16	14.5^{a}±0.12	11.26^{a}±0.10	10.35 ^{b} ± 0.31	12.59 ^{a} ± 0.2 8	0.002
Jejunum	VH (µm)	740.6 ^{c} ±0.76	736 ^{c} 5 ±0.81	792.1^{a}±0.73	764.2^{b}±0.62	746.4 ^{c} ± 0.74	781.3^{b} ± 0.27	0.001
	CD (µm)	75.8^{a} ± 0.38	74.31^{c} ± 0.29	62.14^{b}±0.63	69.71^{a}±0.32	72.54 ^{d} ± 0.41	63.29 ^{b} ± 0.47	0.000
	VH:CD	9.98 ^{b} ± 0.02	10.24 ^{d} ±0.04	12.09^{a}±0.01	11.23^{b}±0.02	10.54 ^{c} ± 0.03	13.01 ^{a} ± 0.02	0.001
Ilium	VH (µm)	545.26 ^{d}±0.96	542.60^{c}±0.61	624.68^{a}±0.64	609.28 ^{c} ±0.40	570.65^{c} ± 0.53	620.61 ^{b} ±0.24	0.001
	CD (µm)	65.45^{a} ± 0.30	63.26 ^{d} ±0.53	47.23^{c}±0.60	50.51^{b} ± 0.65	60.54 ^{d} ± 0.48	49.79 ^{b} ± 0.51	0.002
	VH:CD	9.36^{c}± 0.11	8.98^{c} ± 0.13	13.6^{a}±0.09	11.62 ^{c}± 0.08	9.76 ^{d}± 0.10	12.4 ^{b}± 0.11	0.000

Effect of Vitamin E, Selenium supplementation on intestinal morphology of Japanese Quails intoxicated with Arsenic, Means in the row bearing (a,b,c,d) differ significantly at P >0.05

TABLE 6. Effect of Heavy metals toxicity and supplementation of Vit-E and Se on histo- morphometric characteristics of breast muscle in 35 days old Japanese Quails

Parameters	Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	Pooled S.E.M	P-value
MF-diameter (µm)	314 ^{b} ± 9.26	188.35^{ab}±6.09	191.1^{a} ± 5.35	159.47^{b}±3.24	169.92^{ab}±4.73	188.35^{b} ±6.09	1.72	0.326
MF-area (μm^{2})	215.56^{b}±7.53	107.91^{a} ± 3.59	114.54^{ab}±2.26	107.08^{a}± 2.44	96.99^{b} ± 2.36	107.91^{ab}±3.59	2.293	0.733
MF-density (no.mm^{2})	314 ^{ab} ± 9.26	188.35^{b} ±6.09	191.1 ^{a} ± 5.35	159.47^{b}±3.24	169.92^{ab}±4.73	188.35^{a}±6.09	0.985	0.958
Area of MF/mm^{2}	314^{b}± 9.26	188.35^{ab}±6.09	191.1^{a}± 5.35	159.47^{b}± 3.24	169.92^{ab}±4.73	188.35^{b}± 6.09	1.72	0.326
Area of CT/ mm^{2}	215.56^{b}±7.53	107.91^{a}± 3.59	114.54^{ab}±2.26	107.08^{a}± 2.44	96.99 ^{b}± 2.36	107.91^{ab}±3.59	2.293	0.733

The numeric values with different alphabet superscript^{a-b} in a horizontal table line are statistically different ($p<0.05$). Each number in box is mean of eight replicates. MF: Muscle Fiber, Se: Selenium, As: Arsenic

TABLE 7. Effect of Heavy metals toxicity and supplementation of Vit-E and Se On breast muscle pH and water holding capacity of 35 days old Japanese Quails.

Parameters	Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	P-value
pH-0H	6.26 ^{b}	6.35 ^{b}	6.14 ^{ab}	6.48 ^{a}	6.51 ^{b}	6.11 ^{ab}	0.20
pH-24H	5.69^{b}	5.76^{ab}	5.84^{a}	5.81^{a}	5.88^{ab}	5.37^{a}	0.02
WHC (Drip loss%)	3.74 ^{ab}	2.88 ^{b}	2.98 ^{b}	2.86 ^{b}	2.57 ^{a}	2.34 ^{a}	0.09

The numeric values with different alphabet superscript^{a-b} in a horizontal table line are statistically different ($p<0.05$). Each number in box is mean of five replicates.
WHC: water holding capacity, Se: Selenium, As: Arsenic

TABLE 8. Effect of Heavy metals toxicity and supplementation of Vit-E and Se On Histo- morphometric parameters of Bursa of Fabricius in 35 days old Japanese Quails.

Parameters	Negative Control	Positive Control	Se+Vit.E	As+ Vit. E	As+ Se	As+Se+Vit.E	Pooled S.E.M	P-value
Lymphoid Follicle height (µm)	314 ^{b}± 9.26	188.35^{ab} ± 6.09	191.1^{a} ± 5.35	159.47^{b}±3.24	169.92^{ab}±4.73	188.35^{b} ±6.09	1.72	0.326
Lymphoid Follicle width (µm)	215.56^{b}±7.53	107.91^{a} ± 3.59	114.54^{ab}±2.26	107.08^{a}±2.44	96.99^{b} ±2.36	107.91^{ab}±3.59	2.293	0.733
Lymphoid Follicle area (mm$^{2)}$	314 ^{ab}± 9.26	188.35 ^{b} ± 6.09	191.1^{a} ± 5.35	159.47^{b}±3.24	169.92^{b}±4.73	188.35 ^{a}±6.09	0.985	0.958

The numeric values with different alphabet superscript^{a-b} in a horizontal table line are statistically different ($p<0.05$). Each number in box is mean of eight replicates.
WHC: water holding capacity, Se: Selenium, S.I: Small Intestine, As: Arsenic

TABLE 9. Gross and microscopic lesions recorded in Japanese Quails administered arsenic, vitamin E, Selenium alone and in combination.

Organ	Gross/Microscopic	Lesions	NCG	PCG	Vitamin E + Se	As + Vit E	As + Se	As + Vit E + Se
Liver	Microscopic	Infiltration of leukocytes	+ +	+ +	+ + +	+	+	-
		Sinusoidal spaces	+ +	+ +	+ + +	+	+	-
		Pyknotic nuclei	+ +	+ +	+ + +	+	+	-
		Vacuolar degeneration	+ +	+ +	+ + +	+	+	-
							+	-
Kidneys	Gross	Swollen kidneys	+ +	+ +	+ + +	+	+ +	-
	Microscopic	Vacuolation	+ +	+ +	+ + +	+	+ +	-
		Congestion	+ +	+ +	+ + +	+	+	-
		Glomerular Shrinkage	+ +	+ +	+ + +	+	+ +	-
		Tubular Necrosis	+ +	+ +	+ + +	+	+ +	-
Lungs	Gross	Hemorrhages	+ +	+ +	+ + +	+	+ +	+
		Froth and exudate	+ +	+ +	+ + +	+	+ +	+
	Microscopic	Emphysema	+ +	+ +	+ + +	+	+ +	-
		Congestion	+ +	+ +	+ + +	+	+ +	-
		Edema	+ +	+ +	+ + +	+	+ +	-

Lesion categorization: No lesion (–), mild (+), moderate (++), and severe (+++). As, Arsenic, Vit E: Vitamin E, Se: Selenium. NCG: Negative Control Group, PCG: Positive Control Group, Vitamin E + Se : Vitamin E and Selenium, As + Vit E: Arsenic and vitamin E, As + Se: Arsenic and Selenium, As + Vit E + Se: Arsenic vitamin E and Selenium were having well preserved lobular pattern.

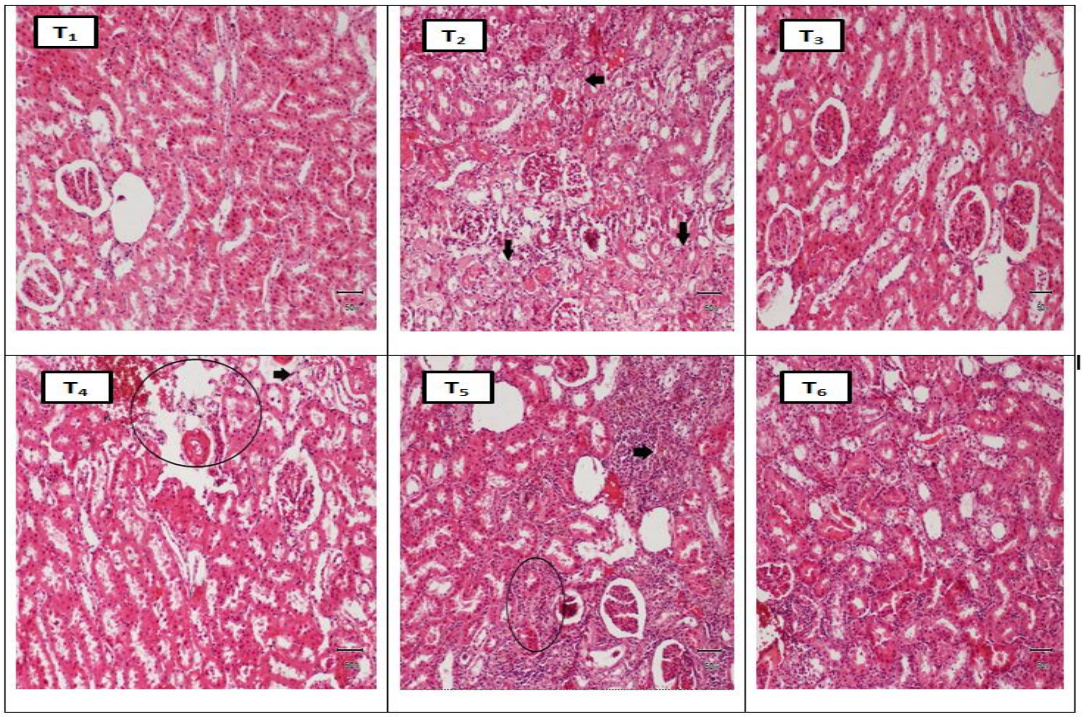

Fig.1. H&E stained Photomicrograph of kidneys of Japanese quails (scale bar = 50)

T_1: Normal histological structure was observed in control group T_2: group (arsenic treated) showing disintegrated cells of basement membrane with pyknosis. Vacuolar degeneration is also observed T_3: showing no microscopic lesions and well-preserved histological structure T_4: group showing Less amount of congestion and low degree of degenerative changes E T_5: Showing disintegrated cells of basement membrane with pyknosis T_6: group showing intact histological structure with less degree of microscopic lesions.

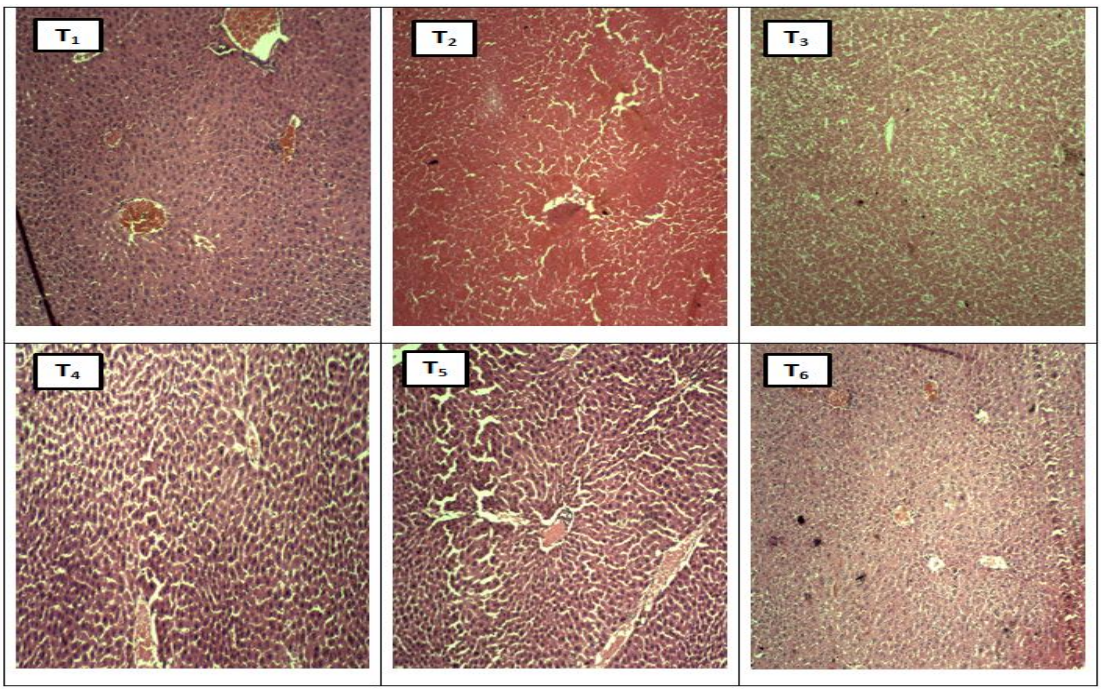

Fig. 2. H&E stained Photomicrograph of the liver of Japanese quails (scale bar = 50).
T_1: control group hepatocytes have well-preserved pattern of lobules and no microscopic lesions observed T_2: group (arsenic treated) hepatocytes showing fatty change with vacuolar degeneration and congestion T_3: group showing well-preserved lobular pattern and no microscopic lesions T_4: group showing congestion and vacuolar degeneration (arrowheads) T_5: Histomicrograph of this group showing vacuolar degeneration and congestion in renal tubular areas T_6: In this group histological section is showing intact lobular pattern and very low or minor level of congestion among hepatocytes.

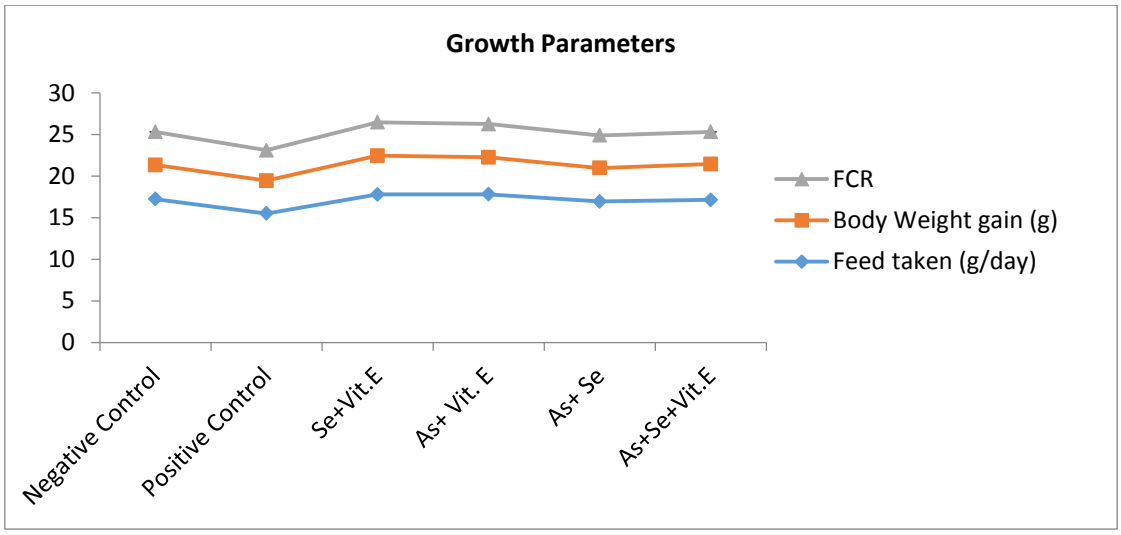

Fig.3. Growth Performance Parameters

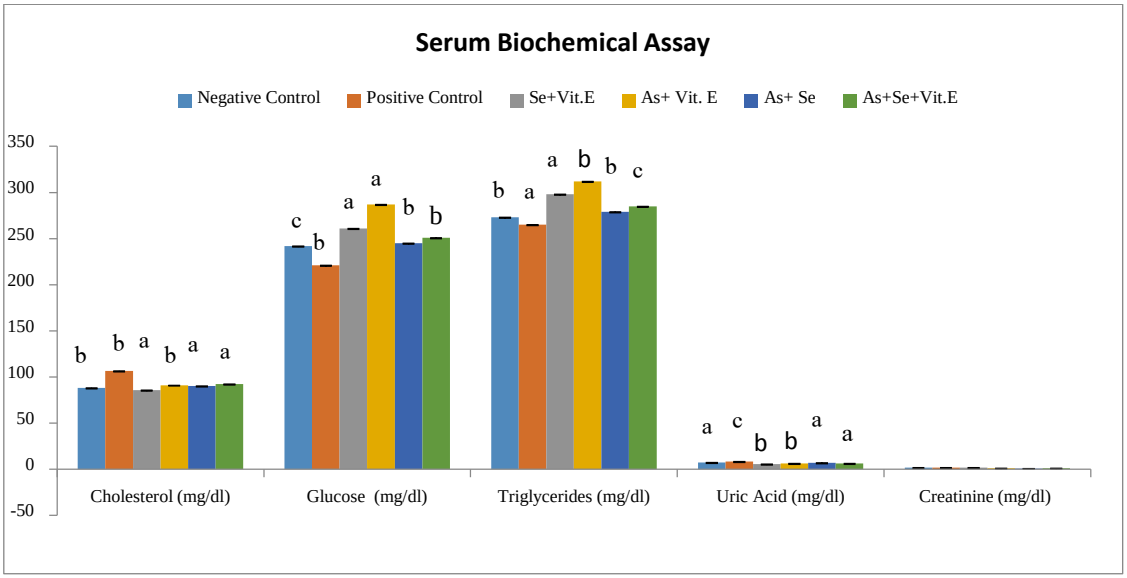

Fig.4. Serum Biochemical Analysis

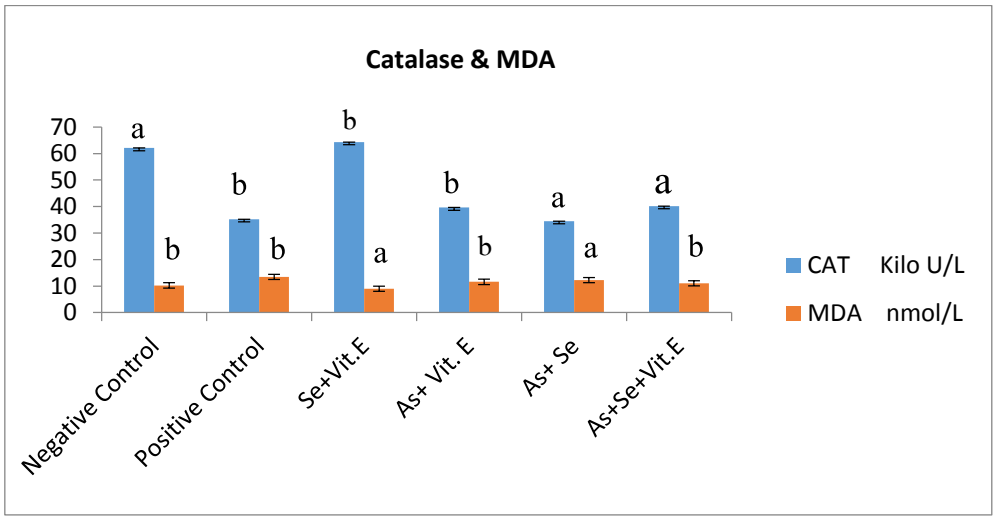

Fig.5. Serum Antioxidant Enzymes

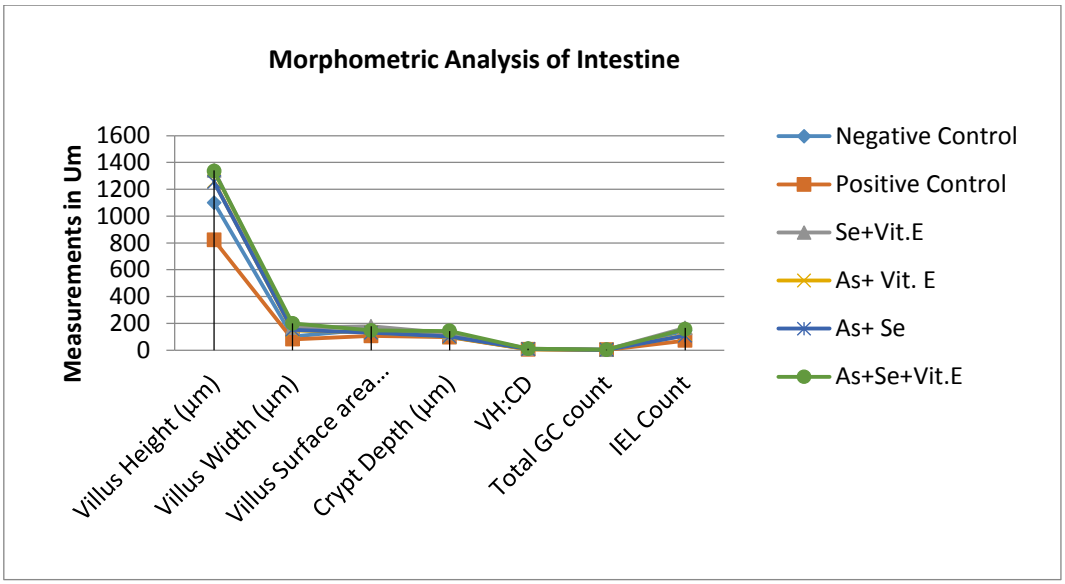

Fig.6. Intestinal Villus Height, Width and VH:CD

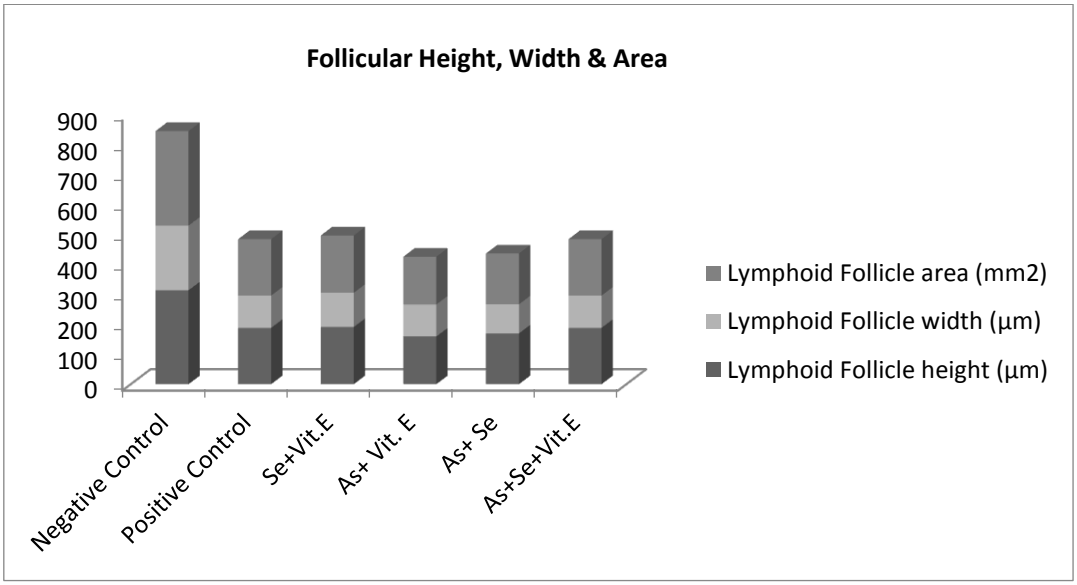

Fig.7. Lymphoid Follicular Height, Width & Area

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