

Biochemical and haematological parameters in experimentally hydro-pericardium syndrome infected broiler chickens supplemented with vitamin E

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ABSTRACT

Hydropericardium syndrome (HPS) was induced experimentally in broiler chickens to assess the role of vitamin E (vit E) supplementation on biochemical and haematological parameters. Serum biochemical studies revealed a significant reduction in serum total protein concentration in both the HPS infected groups but reduction was lower in magnitude in vit E supplemented HPS infected group as compared to HPS infected without vit E supplemented group. Significant reduction in serum albumin concentration was observed in HPS infected without vit E supplemented group. Significant increase in the serum alanine transaminase (ALT) activity was noticed in both the infected groups but was of lower magnitude in vit E supplemented HPS infected group. A significant increase in serum creatine phosphokinase (CPK) activity was observed in HPS infected without vit E supplemented chickens. Haematological studies revealed a significant reduction in haemoglobin (Hb) and packed cell volume (PCV) in HPS infected without vit E supplemented chickens from days 4 to 6 PI. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) values were indicating microcytic normochromic anemia. It is concluded that supplementation of vit E @ 150 mg/kg of feed showed improvement in anemia along with protective effect on biochemical functions of various organs particularly liver and heart.

Key words: Biochemical studies, Chickens, Haematological studies, Hydropericardium syndrome, Vitamin E supplementation

Hydropericardium syndrome (HPS), a viral disease of 3 to 6 week-old broiler chicks is one of the important poultry diseases, having mortality rate of 20–75% (Kataria *et al.* 1997). The aetiological agent of HPS fowl adenovirus (FAV) serotype-4 (Dahiya *et al.* 2002). In Haryana, HPS was reported in the different districts of the state (Mittal 2007). Vit E acts as a powerful antioxidant (Mellors and Tappel 1966) and is essential in maintaining cell membrane integrity of the cardiovascular system (Stephens *et al.* 1996, Food and Nutrition Board 2000, Dickinson 2002, Jialal and Deveraj 2003). Dietary supplementation of vit E also enhances the bird's resistance to various infections in chickens such as *Escherichia coli* (Heinzerling *et al.* 1974), coccidiosis (Colnago *et al.* 1984) and infectious bursal disease (McIlroy *et al.* 1993). Many workers reported a significant increase in the levels of blood urea nitrogen, AP, ALT and CPK in serum of broiler chicks following experimental infection with FAV-

4 (Kumar and Kharole 2001). Some workers also noticed a marked reduction in haemoglobin, packed cell volume and total RBC count in HPS affected chicks (Kumar and Kharole 2001, Nehra *et al.* 2008). The objective of the present study was to assess the effect of vit E supplementation on biochemical and haematological parameters in experimentally HPS infected broiler chicks.

MATERIALS AND METHODS

Experimental chicks and feed: Day-old commercial broiler chicks were procured from a local hatchery having no earlier history of occurrence of HPS in the parent flock. All the birds were given *ad lib.* aflatoxin free standard chick feed and provided clean drinking water throughout the experiment. The approval for conducting the experiment was taken from the Institutional Animal Ethics Committee (IAEC), CCSHAU, Hisar.

Vitamin E (Tocopherol): Vitamin E (tocopheryl acetate) was fed to broilers throughout the experiment after thorough mixing with poultry feed @ 150 mg/kg feed.

Experimental design: Broiler chicks (110) 14-day old were divided randomly into groups A and B of 55 birds each.

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All the birds of group A were given feed mixed with vitamin E @ 150 mg/kg feed throughout the experiment, while the birds of group B were kept on feed without vitamin E supplementation. Each group was further divided into 2 subgroups (group A into A₁ and A₂ and group B into B₁ and B₂) of 35 and 20 birds, respectively. Each bird of group A₁ and B₁ were injected with 0.5 ml of the 10⁻⁴ diluted HPS virus infected liver inoculum (the infective dose, ID₅₀), subcutaneously. ID₅₀ was calculated by a pilot experiment using the standard method (Reed and Muench 1938). Blood samples were collected from each subgroups on 2nd, 4th, 6th and 9th day post infection in sterile tubes for serum separation for biochemical studies and separately in sterile vials containing 2 mg/ml of ethylene diamine tetra acetic acid (EDTA) for haematological studies.

Serum samples were analyzed for total protein concentrations (Tietz 1986a), albumin concentration (Doumas *et al.* 1972), aspartate transaminase activity (Tietz 1986b), alanine transaminase activity (Wroblewski and La Due 1956), creatine phosphokinase activity (Tietz 1986b) by using single step reagent kits.

Haemoglobin concentration, packed cell volume, total erythrocyte count were estimated by cyanmet, microhaematocrit and improved Neubauer Haemocytometer methods (Benjamin 1978), respectively. Mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) were calculated on the basis of the values of Hb, PCV and TEC.

Statistical analysis of data: The data for various haematological and biochemical parameters were subjected to statistical analysis by using analysis of variance technique (Snedecor and Cochran 1967).

RESULTS AND DISCUSSION

Hydropericardium syndrome (HPS) could be produced in the chicks of groups A₁ and B₁ on day 2 PI as evidenced by characteristic posture with closed eyes and chest and beak resting on the ground (Fig. 1) and hydropericardium giving litchi like appearance (Fig. 2).



Figs 1–2. 1. Chicken from HPS infected group exhibiting severe depression and sitting in “chest resting posture” with closed eyes. 2. Chicken from HPS infected without vitamin E supplemented group showing accumulation of serous fluid in the pericardial sac (hydropericardium).

Biochemical studies

Mean values of biochemical parameters in different experimental groups are given in Table 1. A significant reduction in total protein concentration was noticed in both the infected groups as compared to control from days 2 to 4 PI. A significant reduction in serum albumin concentration was observed in HPS infected without vit E supplemented group as compared to control from days 2 to 4 PI. Whereas, mean serum albumin concentrations of both non-infected groups were more or less similar from days 2 to 6 PI. Significant reduction in serum total protein concentration was observed only in HPS infected without vit E supplemented group by other workers in broiler chicks due to HPS infection (Kumar and Kharole 2001, Rawat *et al.* 2010). Hypoalbuminaemia observed in present study might be attributed to hepatitis/hepatic necrosis, which is a consistent finding in HPS (Rawat *et al.* 2010) and was observed in present study too. In vit E supplemented HPS infected group, this reduction in protein was significantly less.

A significant increase in serum alanine transaminase (ALT) activity was observed in both the infected groups as compared to control from days 4 to 6 PI. This increase in serum ALT activity was lower in vit E supplemented HPS infected group as compared to HPS infected without vit E supplemented group throughout the experiment. Increase in serum aspartate transaminase (AST) activity was observed in HPS infected without vit E supplemented group as compared to control from days 2 to 6 PI though it was non-significant, whereas this increase in serum AST activity was significant on day 9 PI. Serum ALT results are in accordance with the findings of other workers in natural and experimental cases of HPS affected chicks (Rawat *et al.* 2010, Kumar and Kharole 2001). Since ALT is present in large quantities in hepatocytes, the increased activity of ALT might be because of excessive release of this enzyme from liver as a result of hepatic necrosis/hepatitis which has been noticed in HPS infected groups of the present study. This increase in ALT activity was relatively less in vit E supplemented HPS infected group indicating hepatoprotective effect of vit E supplementation. A significant increase in serum creatine phosphokinase (CPK) activity was observed in HPS infected without vit E supplemented group as compared to control from day 4 PI onwards. More or less similar results regarding serum AST and CPK activity were reported in natural and experimental cases of HPS in the literature (Rawat *et al.* 2010, Kumar and Kharole 2001, Deepak *et al.* 2001). Since this enzyme is normally present in large quantities in cardiac muscles (Benjamin 1978), the increased activity of these enzymes in serum might be correlated to cardiac lesions. The quantitative differences in the results of serum CPK activity among both the infected groups indicate cardioprotective effect of vit E supplementation. Vitamin E supplementation alone did not show any significant effect on serum biochemical activities.

Table 1. Mean values of different biochemical parameters in serum of broiler chicks infected with HPS and supplemented with vitamin E (mean±SE)

Parameters	Groups	Post infection period (days)				
		0	2	4	6	9
Total protein concentration (g/dl)	Control	5.91±0.21 ^a	5.89±0.21 ^a	5.90±0.14 ^a	5.47±0.14 ^a	5.40±0.26 ^a
	Vit E	5.89±0.15 ^a	5.91±0.14 ^a	5.69±0.12 ^a	5.49±0.17 ^a	5.78±0.30 ^a
	HPS	5.91±0.21 ^a	4.98±0.40 ^b	2.93±0.64 ^b	4.85±0.30 ^{ab}	5.82±0.11 ^a
	Vit E + HPS	5.89±0.15 ^a	5.09±0.08 ^b	3.15±0.75 ^b	4.38±0.47 ^b	5.83±0.18 ^a
Albumin concentration (g/dl)	Control	2.47±0.21 ^a	2.19±0.06 ^a	2.29±0.13 ^a	2.04±0.04 ^a	1.70±0.08 ^b
	Vit E	2.32±0.14 ^a	2.18±0.09 ^a	2.05±0.04 ^a	2.18±0.03 ^a	2.05±0.07 ^a
	HPS	2.47±0.21 ^a	1.94±0.06 ^b	1.58±0.21 ^b	2.01±0.11 ^a	1.97±0.10 ^a
	Vit E + HPS	2.32±0.14 ^a	2.19±0.07 ^a	2.08±0.03 ^a	2.38±0.30 ^a	2.06±0.04 ^a
Alanine transaminase activity (IU/L)	Control	7.48±0.27 ^a	7.52±1.06 ^a	9.01±0.20 ^{ab}	6.94±0.57 ^b	8.02±0.61 ^a
	Vit E	7.19±0.45 ^a	7.10±0.73 ^a	6.83±0.75 ^b	5.86±0.43 ^b	7.16±0.56 ^a
	HPS	7.48±0.27 ^a	7.65±0.67 ^a	12.41±1.91 ^a	11.49±1.31 ^a	8.28±0.53 ^a
	Vit E + HPS	7.19±0.45 ^a	7.68±0.77 ^a	12.25±1.98 ^a	11.15±1.89 ^a	7.73±0.58 ^a
Aspartate transaminase activity (IU/L)	Control	273.18±17.48 ^a	282.30±21.33 ^a	261.70±16.48 ^a	297.10±29.65 ^{ab}	264.96±14.89 ^{bc}
	Vit E	255.00±12.75 ^a	257.62±24.86 ^a	248.52±13.61 ^a	256.98±20.26 ^b	221.90±13.26 ^c
	HPS	273.18±17.48 ^a	317.60±20.90 ^a	300.32±22.71 ^a	337.28±20.55 ^a	323.36±20.03 ^a
	Vit E + HPS	255.00±12.75 ^a	260.52±13.15 ^a	253.54±18.72 ^a	291.66±28.33 ^{ab}	274.30±12.62 ^b
Creatine phosphokinase activity (IU/L)	Control	213.58±10.17 ^a	270.98±14.24 ^a	246.30±17.89 ^b	278.06±22.40 ^b	274.4±23.83 ^b
	Vit E	210.94±6.24 ^a	257.42±34.37 ^a	254.34±19.00 ^b	302.80±31.34 ^b	290.78±27.27 ^{ab}
	HPS	213.58±10.17 ^a	221.00±21.90 ^a	447.66±29.57 ^a	425.00±15.76 ^a	360.76±24.34 ^a
	Vit E + HPS	210.94±6.24 ^a	213.40±26.63 ^a	329.10±49.19 ^b	313.12±49.19 ^b	239.44±18.60 ^b

a, b, c: Means with unlike superscript letters in a column are significantly different at $P < 0.05$.

Haematological studies

Mean values of haematological parameters in different experimental groups are given in Table 2. Significant decrease in haemoglobin (Hb) concentration was observed in both the infected groups as compared to control from days 4 to 6 PI. However on day 4 PI, this decrease was significantly more in the HPS infected without vit E supplemented group as compared to the vit E supplemented HPS infected group. Packed cell volume (PCV) values were significantly lower in HPS infected without vit E supplemented group as compared to control from day 4 PI onwards. Reduction in total erythrocyte count (TEC) was observed in both the infected groups as compared to control throughout the experiment though it was nonsignificant.

Values of mean corpuscular volume (MCV) were significantly lower in HPS infected without vit E supplemented group as compared to control from days 4 to 6 PI indicating microcytic anemia due to HPS infection. MCV value of HPS infected vit E supplemented group was significantly more than vit E supplemented non-infected group on day 9 PI. Difference in the values of mean corpuscular haemoglobin concentration (MCHC) between HPS infected without vit E supplemented group and control was nonsignificant from days 2 to 4 PI indicating normochromic anemia due to HPS infection. Vitamin E supplementation alone did not show any significant effect on the haematological values.

Reduction in Hb, PCV and TEC in the present study is suggestive of anemia. Almost similar findings were reported by other workers in experimental and natural cases of HPS (Kumar and Grewal 2008, Kumar and Kharole 2001). Internal haemorrhages in cardiac muscles and other organs of HPS infected groups might have caused reduction in Hb concentration and TEC. MCV and MCHC values showed that the anemia was microcytic normochromic type on days 4 and 6 PI. It is concluded that supplementation of vitamin E @ 150 mg/kg of feed showed improvement in anemia along with protective effect on biochemical functions of various organs particularly liver and heart.

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Table 2. Mean values of different haematological parameters of broiler chicks infected with HPS supplemented with vitamin E (mean±SE)

Parameters	Groups	Post infection period (days)				
		0	2	4	6	9
Hb (g/dl)	Control	6.80±0.30 ^a	7.68±0.41 ^a	7.20±0.14 ^a	7.64±0.27 ^a	7.04±0.26 ^a
	Vit E	7.68±0.35 ^a	7.72±0.53 ^a	7.28±0.10 ^a	7.08±0.19 ^a	6.92±0.29 ^a
	HPS	6.80±0.30 ^a	6.72±0.19 ^a	6.04±0.12 ^c	5.76±0.26 ^b	6.92±0.21 ^a
	Vit E + HPS	7.68±0.35 ^a	7.08±0.66 ^a	6.56±0.21 ^b	6.08±0.26 ^b	6.92±0.19 ^a
PCV (%)	Control	26.40±1.36 ^a	28.00±1.79 ^a	29.40±2.64 ^a	28.80±1.80 ^a	30.20±1.36 ^a
	Vit E	26.40±1.21 ^a	28.00±1.05 ^a	25.80±1.70 ^a	27.60±0.40 ^a	26.80±0.80 ^b
	HPS	26.40±1.36 ^a	24.40±1.97 ^a	20.20±0.80 ^b	21.60±0.51 ^b	26.40±0.51 ^b
	Vit E + HPS	26.40±1.21 ^a	24.20±0.66 ^a	24.60±0.98 ^a	27.60±0.93 ^a	29.00±1.27 ^{ab}
TEC(million/μl)	Control	2.63±0.17 ^a	2.43±0.21 ^a	2.43±0.15 ^a	2.34±0.19 ^a	2.46±0.09 ^a
	Vit E	2.38±0.15 ^a	2.28±0.15 ^a	2.42±0.14 ^a	2.05±0.17 ^a	2.57±0.20 ^a
	HPS	2.63±0.17 ^a	2.36±0.13 ^a	2.40±0.07 ^a	2.15±0.08 ^a	2.53±0.05 ^a
	Vit E + HPS	2.38±0.15 ^a	1.99±0.20 ^a	2.25±0.04 ^a	2.11±0.08 ^a	2.06±0.11 ^b
MCV (fl)	Control	101.94±7.91 ^a	119.28±13.38 ^a	121.77±11.19 ^a	126.51±12.28 ^a	123.91±9.44 ^{ab}
	Vit E	113.82±11.74 ^a	125.33±10.37 ^a	108.65±10.38 ^a	139.25±14.60 ^a	94.30±16.66 ^c
	HPS	101.94±7.91 ^a	104.79±11.53 ^a	84.32±3.5 ^b	101.05±3.98 ^b	104.39±2.46 ^{bc}
	Vit E + HPS	113.82±11.74 ^a	125.68±10.06 ^a	109.38±4.28 ^a	131.38±5.30 ^a	141.25±2.87 ^a
MCHC (%)	Control	26.10±2.13 ^a	27.66±1.60 ^a	25.41±2.60 ^b	26.82±1.48 ^a	23.45±1.12 ^a
	Vit E	29.36±1.99 ^a	27.87±2.63 ^a	28.70±1.92 ^{ab}	25.65±0.53 ^{ab}	25.98±1.62 ^a
	HPS	26.10±2.13 ^a	28.42±2.81 ^a	30.10±1.42 ^b	26.78±1.58 ^a	26.30±1.21 ^a
	Vit E + HPS	29.36±1.99 ^a	29.71±2.53 ^a	26.77±1.05 ^b	22.12±1.21 ^b	24.08±1.39 ^a

Hb, Haemoglobin; PCV, Packed cell volume; TEC, total erythrocyte count; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; a, b, c, means with unlike superscript letters in a column are significantly different at P < 0.05.

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