Changes in Serum Protein Profile, Cholesterol and Blood Glucose during Endotoxic Shock in Buffalo Calves Supplemented with Vitamin E and Selenium

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ABSTRACT : A study was conducted to monitor the changes in serum protein profile, cholesterol and blood glucose during endotoxic shock in buffalo calves and also to assess the role of prophylactic supplementation of vitamin E and selenium in alleviating the endotoxic effects. Fifteen male buffalo calves (6-8 months of age) were divided into three groups: Group I (control)-infused with 0.9% saline solution; Group II-infused with E. coli endotoxin at 5 μg/kg body weight in normal saline solution; Group III- supplemented prophylactically with 250 mg vitamin E and 7.5 mg selenium by i/m injections at weekly intervals for one month prior to the induction of endotoxic shock. The blood samples were collected at 0, 1, 3, 6, 9, 12, 24, 48 and 72 h after the induction of shock. Endotoxin caused a significant ($p<0.05$) hypoproteinemia from 3-12 h post infusion in group II but this hypoproteinemia was less pronounced and only from 3-9 h post infusion in vitamin E and selenium supplemented calves. Hypoglycemia was observed in group II from 3-24 h and blood glucose level returned to normal at 72 h. However hypoglycemia was mild in group III and blood glucose returned to normal at 48 h. Hypocholesterolaemia and hypoalbuminemia were found in both groups II and III but these changes were less pronounced in group III i.e. vitamin E and Se supplemented calves. Serum electrophoretic protein patterns of group III were quite similar to those of control group but animals of group II had different electrophoretic pattern. It was concluded that the antioxidant effects of vitamin E and Se prevent the liver against oxidative stress during endotoxic shock. ***(Asian-Aust. J. Anim. Sci. 2005. Vol 18, No. 2 : 192-196)***

Key Words : Endotoxin, Vit. E and Se, Serum Biochemical Changes, Buffalo Calves

INTRODUCTION

Endotoxic shock has been found to occur frequently in ruminants especially cattle and buffalo (Constable et al., 1993). Endotoxin gains access into circulation due to dysfunctions in normal protective barriers within the body and causes production of free radicals resulting into severe cellular damage (Portoles et al., 1996), particularly to the important organs like liver, kidneys, brain and myocardium. Antioxidants, most importantly, vitamin E and selenium, directly protect the cell membranes from free radical damage. Prophylactic supplementation of vitamin E and Se had been successful in reducing the quantum of oxidative damage due to formation of free radicals during endotoxic shock (Sandhu and Singha, 2003). Liver is the major organ involved in the synthesis of serum proteins. Damage to liver during endotoxic shock, thus, may results in alterations in the profiles of these metabolites in the blood. Therefore this study was planned to monitor the changes in serum protein profile, cholesterol and blood glucose before and after induction of endotoxic shock in buffalo calves with/without prophylactic supplementation of vitamin E and selenium.

MATERIALS AND METHODS

Fifteen male buffalo calves (aged 6-8 months) selected for this study were procured from the local market and were maintained as per standard management and feeding conditions practiced at the dairy farm, P. A. U. Ludhiana. The animals were kept under semi loose housing conditions with half walled open sheds and concrete floors. Throughout the experimental period, the animals were given adequate amount of chaffed/unchaffed green fodder along with wheat straw. Drinking water was available round the clock.

The calves were divided into three groups of five animals each. Group I served as control and the animals in this group were administered with normal saline solution (NSS) intravenously (i/v). Group II comprised animals in which endotoxic shock was induced experimentally. Each animal in this group was infused with E. coli endotoxin at 5 g/kg body weight. Total dose was calculated as per body weight of the calves, dissolved in 100 ml. NSS and infused by a slow i/v injection over a period of half-hour. The endotoxin used was lyophilized phenol extracted E. coli endotoxin 0111: B4 lipopolysaccharide that was procured from SIGMA Chemicals, USA.

Group III served as treatment group. Each animal of this group received a prophylactic supplementation of 250 mg vitamin E and 7.5 mg selenium as intramuscular (i/m) injection E–care–Se Vet (Vetcare Ltd., Bangalore). Only then these calves were subjected to endotoxic shock which was induced by giving E. coli endotoxin at the same dose rate as in Group II.

The blood samples were collected aseptically from the jugular vein in stoppered collection vials. In Groups II and III, the samples were taken at 1, 3, 6, 9, 12, 24, 48 and 72 h after the induction of endototoxic shock and one normal

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Table 1. Effect of endotoxic shock on serum total proteins, albumin, globulins and immunoglobulins in buffalo calves with prophylactic vitamin E and Se supplementation

Group	Time (hours)								
	0 h	1 h	3 h	6 h	9 h	12 h	24 h	48 h	72 h
	Total serum proteins (g/dL)								
Group I	7.35±0.34[a]	7.20±0.25[a]	7.64±0.27[a]	7.20±0.21[a]	7.37±0.20[a]	7.65±0.45[a]	7.33±0.46[a]	7.52±0.42[a]	7.30±0.30[a]
Group II	7.40±0.43[a]	7.78±0.45[a]	6.10±0.30*[b]	5.62±0.47*[b]	5.22±0.36*[b]	5.94±0.37*[b]	7.22±0.48[a]	7.34±0.41[a]	7.22±0.47[a]
Group III	7.08±0.38[a]	7.38±0.35[a]	6.74±0.57[b]	6.56±0.46[b]	6.46±0.34[b]	6.74±0.30[b]	6.96±0.41[a]	7.04±0.34[a]	7.06±0.35[a]
	Albumin (g/dL)								
Group I	3.60±0.20[a]	3.50±0.30[a]	3.70±0.20[a]	3.40±0.20[a]	3.60±0.20[a]	3.60±0.30[a]	3.60±0.20[a]	3.70±0.20[a]	3.60±0.30[a]
Group II	3.40±0.40[a]	3.00±0.40[a]	2.60±0.10*[b]	2.30±0.20*[b]	2.20±0.10*[b]	2.40±0.10*[b]	3.10±0.10[a]	3.40±0.30[a]	3.20±0.30[a]
Group III	3.40±0.20[a]	3.00±0.20[a]	2.80±0.20[b]	2.70±0.10[b]	2.70±0.20[b]	3.30±0.20[b]	3.40±0.30[a]	3.50±0.10[a]	3.50±0.30[a]
	Globulins (g/dL)								
Group I	2.90±0.10[a]	2.80±0.10[a]	3.10±0.20[a]	3.00±0.20[a]	2.90±0.20[a]	3.10±0.30[a]	2.90±0.10[a]	3.00±0.20[a]	2.90±0.10[a]
Group II	3.20±0.50[a]	3.40±0.40[a]	2.40±0.40[a]	2.30±0.50[a]	2.40±0.60[a]	2.60±0.60[a]	3.20±0.60[a]	3.20±0.70[a]	3.10±0.80[a]
Group III	2.90±0.50[a]	3.00±0.50[a]	2.50±0.70[a]	2.50±0.30[a]	2.30±0.60[a]	2.60±0.70[a]	2.80±0.40[a]	2.80±0.30[a]	2.80±0.50[a]
	Immunoglobulins (g/dL)								
Group I	0.78±0.13[a]	0.78±0.13[a]	0.80±0.14[a]	0.77±0.15[a]	0.80±0.14[a]	0.79±0.15[a]	0.79±0.13[a]	0.78±0.20[a]	0.78±0.15[a]
Group II	0.80±0.06[a]	0.80±0.10[a]	0.83±0.08[a]	0.84±0.17[a]	0.85±0.20[a]	0.83±0.15[a]	0.86±0.20[a]	0.80±0.15[a]	0.83±0.15[a]
Group III	0.75±0.15[a]	0.80±0.16[a]	0.76±0.14[a]	0.80±0.15[a]	0.75±0.14[a]	0.73±0.09[a]	0.73±0.14[a]	0.73±0.15[a]	0.75±0.14[a]

Group I: Control. Group II: Endotoxic shock. Group III: Vitamin E and Se+endotoxic shock.
Values in columns with same superscripts have no significant difference ($p<0.05$).
Values in rows having asterisk differ significantly from the normal values ($p<0.05$).

sample (0 h) before the induction of shock. Similar schedule was followed for the control group i.e. before and after the infusion of NSS alone. One ml. of blood sample was used for the preparation of protein free filtrate for estimating blood glucose. Serum was separated from the rest of blood and stored in a deep freezer in different aliquots for analysis of various biochemical parameters.

Blood glucose was estimated by the method of Folin and Wu (1920). Serum cholesterol and total proteins were analyzed by the modified method of Zak (1957) and biuret method of Reinhold (1953), respectively. Serum albumin was determined by chemistry analyzer (RA-50, Miles India Ltd., Baroda) using autopak albumin kit (Bayer Diagnostics India Ltd., Baroda). Serum immunoglobulins were precipitated by 45% saturated solution of ammonium sulfate. The precipitates were dissolved in phosphate buffer saline and the protein in the suspension was determined (Lowry et al., 1951). SDS-PAGE used for the separation of different protein fractions was done by the method of Laemmli (1970). For statistical analysis the methods detailed by Snedecor and Cochran (1976) were followed.

RESULTS AND DISCUSSION

Serum total proteins:

The normal means of total proteins were 7.35±0.34, 7.40±0.43 and 7.08±0.38 g/dL for control, endotoxic shock and treatment groups, respectively (Table 1). These values were in agreement with those reported by Kumar (1989) but were slightly lower (8.55±0.20 g/dL) than those reported in buffalo calves (Paul and Vadlamudi, 1974). In control group, there was no significant change in the serum proteins levels throughout the study (Table 1). A significant ($p<0.05$) hypoproteinemia was observed from 3-12 and 3-9 h post infusion in endotoxic shock as well as treatment groups (Figure 1), respectively and then the levels returned towards normal.

Total serum protein concentration in endotoxic shock group was significantly lower than that of control group from 3-12 h. This hypoproteinemia was less appreciable in treatment group as compared to endotoxic shock group (Figure 1). The initial non significant hyperproteinemia may be due to the release of some positive acute phase proteins mainly glycoproteins released by hepatocytes upon specific stimulation by cytokines (Gruys et al., 1994). Consequently, a significant hypoproteinemia may possibly be due to increased ability of carbon skeletons of amino acids to enter Kreb's Cycle for increased protein breakdown. Moreover the increased hepatic mRNA synthesis for the positive acute proteins is associated with a dramatic decline in synthesis of serum proteins normally formed, such as serum albumin. During the acute phase response, these proteins show a decline and thus represent the negative acute phase proteins (Heinrich et al., 1990; Sammalkorpi et al., 1990). The decreased ability of anoxic liver to utilize amino acids for protein biosynthesis (Engel et al., 1943) and also the decreased albumin and globulin, as observed (Table 1) during this study was to some extent responsible for hypoproteinemia. Simultaneously, the haemodilution as a result of shift of interstitial fluid in response to shock may also be responsible for hypoproteinemia in buffalo calves (Singh, 1979).

Table 2. Effect of endotoxic shock on blood glucose and serum cholesterol in buffalo calves with prophylactic vitamin E and Se supplementation

Group	Time (hours)								
	0 h	1 h	3 h	6 h	9 h	12 h	24 h	48 h	72 h
	Blood glucose (mg/dL)								
Group I	60.60±3.20^{a}	67.40±4.40^{a}	66.20±3.10^{a}	65.70±4.50^{a}	62.70±3.20^{a}	61.00±3.40^{a}	60.60±3.20^{a}	61.00±2.60^{a}	60.50±3.40^{a}
Group II	62.50±4.50^{a}	68.00±4.50^{a}	54.10±3.50*^{b}	45.60±3.50*^{b}	40.80±2.40*^{b}	42.50±3.00*^{b}	49.40±3.70*^{b}	57.00±3.00^{a}	57.90±3.50^{a}
Group III	61.60±4.80^{a}	66.70±5.20^{a}	50.70±4.60*^{b}	48.50±5.40*^{b}	51.60±5.20*^{c}	51.20±6.20*^{c}	52.00±6.20*^{b}	60.00±5.10^{a}	60.90±4.50^{a}
	Serum cholesterol (mg/dL)								
Group I	88.00±7.10^{a}	87.10±8.20^{a}	88.40±6.60^{a}	88.00±7.10^{a}	86.40±5.70^{a}	87.70±7.30^{a}	90.30±7.00^{a}	87.20±8.00^{a}	87.60±7.20^{a}
Group II	90.50±8.50^{a}	79.90±5.60^{a}	70.50±5.00*^{a}	71.20±6.60*^{a}	71.50±8.50*^{a}	85.60±9.98^{a}	89.50±8.10^{a}	89.60±6.50^{a}	89.90±7.80^{a}
Group III	89.60±8.70^{a}	77.00±6.40^{a}	69.50±4.80*^{a}	70.00±6.60*^{a}	73.80±8.80*^{a}	86.00±8.50^{a}	88.70±9.00^{a}	90.60±8.70^{a}	90.20±8.60^{a}

Group I: Control. Group II: Endotoxic shock. Group III: Vitamin E and Se+endotoxic shock.
Values in columns with same superscripts have no significant difference ($p<0.05$).
Values in rows having asterisk differ significantly from the normal values ($p<0.05$).

Though the vitamin E and selenium treated group also showed hypoproteinemia but it was mild and to a lesser extent as compared to that observed in endotoxic shock group (Figure 1). This may be attributed to the antioxidant effect of vitamin E and selenium preventing the initiation of free radical chain reactions (Mayes, 1996) thereby protecting liver against oxidative stress.

Albumin

The normal means of albumin in control, endotoxic shock and treatment groups were 3.6±0.20, 3.4±0.40 and 3.4±0.20 g/dL, respectively (Table 1). These values were comparable to 3.29±0.13 g/dL reported in cows (Kaneko, 1997). Levels of albumin in group I remained relatively unchanged throughout the experiment. A significant hypoalbuminemia was observed from 3-12 and 3-9 h post infusion, in endotoxic shock and treatment groups (Figure 1), respectively followed by the return of albumin levels towards normal. The hypoalbuminemia in the treatment group was less pronounced as compared to that of endotoxic shock group.

The decline in albumin concentration can be due to either fluid loss or failure of albumin synthesis. Loss of blood and plasma and diarrhoea may lead to hypoalbuminemia (Kaneko, 1997). The sensitivity of albumin synthesis to protein and nitrogen loss, that occurs in any form of diarrhoea further compounds hypoalbuminemia (Kaneko, 1997) leading to a generalized hypoproteinemia. Hepatic anoxia during endotoxic shock may be another reason for decreased albumin in serum because liver is the only site of albumin synthesis.

A less pronounced hypoalbuminemia in the treatment group may be associated with the protective effect of antioxidants vitamin E and selenium on liver by preventing the membrane lipid peroxidation and free radical chain reaction.

Globulins

The normal means of globulins in control, endotoxic shock and treatment groups were 2.9±0.10, 3.2±0.80 and 2.9±0.50 g/dL, respectively (Table 2). These values were in accordance with those reported by Singh (1998) for buffaloes. A non significant hypoglobulinemia was observed in the endotoxic shock and the treatment groups from 3-9 h post infusion, however, there was no significant difference in the globulins levels among the groups, throughout the experiment.

Total immunoglobulins

Normal average values of Ig in control, endotoxic shock and treatment groups were 0.78±0.13, 0.80±0.06 and 0.75±0.15 g/dL, respectively (Table 2). These were comparable to Ig values of 0.87±0.55 in buffaloes (Singh, 1998). The total Ig concentration did not show any marked variations from normal values in all the three groups.

The electrophoretic protein patterns of the calves of endotoxic shock group were different from those of control group. The densities of all the protein fractions increased at 1 h post infusion followed by an appreciable decrease in the densities of almost all the fractions from 3-9 h post-infusion. The densities of the protein fraction of albumin returned to normal thereafter whereas the densities of some fast moving globulin fractions remained lower upto 72 h post-infusion.

The decline in albumin densities may be due either to fluid loss or hepatic anoxia during endotoxic shock as discussed earlier. Though the decrease in total globulins was non-significant as observed in this study, the decrease in some globulin fractions even upto 72 h of study, needs further investigation.

The electrophoretic patterns of proteins of the calves of the treatment group were quite similar to those of control group, thereby indicating that vitamin E and selenium have got some role in minimizing the effect of endotoxic shock on serum protein changes. These findings corroborated with those of Sakaguchi et al. (1981,a) for the membrane electrophoretic profile during endotoxic shock and after α-tocopherol supplementation in endotoxic mice.

Blood glucose

The normal means were 60.6±3.20, 52.5±4.5 and 61.6±4.8 mg/dL for control, endotoxic shock and treatment group, respectively. A non-significant hyperglycemia was observed upto 1-h post infusion in all the groups, followed by reversal of the above trend and returning of level towards the basal value in control group (Table 3).

A significant ($p<0.05$) fall in glucose levels were observed from 3-24 h post infusion in both the endotoxic shock and the treatment groups (Figure 2). However this hypoglycemia was mild in the treatment group and the glucose level returned towards normal at 48 h post infusion while the time taken for endotoxic shock group was 72 h post infusion. The blood glucose levels in both these groups were significantly ($p<0.05$) lower than those in control group from 3-24 h post infusion. However, the blood glucose concentrations in endotoxic shock and the treatment groups showed a significant difference between each other from 9-12 h of endotoxin infusion.

The normal mean values recorded in different groups were comparable to 60.61±2.3 mg/dL, reported in cow calves (Singh and Sodhi, 1992) but were slightly higher than 43.34±4.41 mg/dL as observed by Paul and Vadlamudi (1974). The initial hyperglycemia at I hour of endotoxin infusion may be due to secretion of epinephrine in response to stressful stimuli leading to glycogenolysis in liver and muscles causing higher blood glucose concentration (Singh and Sodhi, 1992). A hypoglycemia in later stages may be attributed to an insulin like activity of endotoxin and an impaired gluconeogenic capability of hepatocytes (Phillips et al., 1981), or due to increased hepatic glucose production (Naylor and Kronfeld, 1985). This bi-phasic pattern of blood glucose levels observed in the present study was in accordance with that observed previously in calves (Singh, 1979; Phillips et al., 1981; Singh, 2000).

Serum cholesterol

The normal means of serum cholesterol in control, endotoxic shock and treatment groups were 88.0±7.1, 90.5±8.5 and 89.6±8.7 mg/dL (Table 3), respectively. There was no significant change in these levels upto 72 h within control group. A significant ($p<0.05$) hypocholesterolaemia from 3-9 h post infusion in both endotoxic shock and the treatment groups was observed (Figure 2). The concentrations achieved normal baseline values thereafter. The normal mean values of serum cholesterol in different groups were comparable to 95.61±2.02 mg/dL (Bhullar, 1998) in buffalo calves but were slightly higher than 64.8±3.57 mg/dL reported by Singh and Sodhi (1992).

A significant ($p<0.05$) hypocholesterolemia induced by the endotoxic shock may be due to the increased production of corticosteroids (to counter the stress caused by endotoxic shock) as cholesterol is the obligatory intermediate in corticosteroid biosynthesis. Moreover, this effect may be associated with increased levels of SGOT during endotoxic shock (Singh and Sodhi, 1992) because the increased SGOT concentration decreases the availability of acetyl CoA for cholesterol synthesis. Since no significant difference was recorded between endotoxic shock and the treatment groups, it might indicate that the supplementation of vitamin E and selenium had no effect on the hypocholesterolaemia induced by endotoxic shock.

The results of this study showed that hypoproteinemia and hypoglycemia were less pronounced during endotoxic shock in the calves supplemented with vitamin E and Se.

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