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Antioxidant potential of coenzyme Q10 in *Escherichia coli* associated calf diarrhea

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ABSTRACT

The aim of the present study was to evaluate the antioxidant potential of coenzyme Q10 in *Escherichia coli* associated calf diarrhea. Six diarrheic calves were treated with standard treatment (ST) and another six diarrheic calves were given standard treatment along with coenzyme Q10 (ST-CoQ10). Whereas, six healthy calves received only placebo. The results revealed a significant reduction of Hb, PCV, TLC, albumin, BUN, creatinine, oxidative stress indicators, IFABP2, fecal consistency score and dehydration (%) in diarrheic calves treated with either ST or ST-CoQ10 on day 3 and 5. However, ST-CoQ10 treatment effectively reduced the oxidative stress indicators and IFABP 2 much earlier (day 3) compared to ST treatment alone. The results of the present study suggest that the incorporation of CoQ10 in standard treatment represents a potential additional treatment option in the case of *E. coli* associated calf diarrhea.

Key words: Calf diarrhea, Coenzyme Q10, *Escherichia coli*, IFABP 2, Oxidative stress

Calf diarrhea is a commonly reported disease and one of the leading cause of neonatal mortality in any dairy herd (Uhde *et al.* 2008, Bartels *et al.* 2010). The etiology of calf diarrhea is multifactorial and include infective, environmental, nutritional, and management factors (Blom 1981, Clement *et al.* 1995, Svensson *et al.* 2003). Among the infectious causes, enterotoxigenic *Escherichia coli* is the most common (Ottosen *et al.* 1959). During the course of pathogenesis, *E. coli* produces numerous types of enterotoxins that cause villous atrophy due to loss of infected cells and damage to the laminar propria of small intestine (Cho *et al.* 2014). Intestinal fatty acid binding protein (IFABP), an intracellular protein (15 kDa) expressed in villus tips of the mature enterocytes of the small intestine, plays an important role in the transportation and metabolism of long-chain fatty acids (Funaoka *et al.* 2010). IFABP is considered to be rapidly released into the circulation just after small intestinal mucosal tissue injury (Windsant *et al.* 2012). Studies demonstrated that IFABP can be used as intestinal injury biomarker in mesenteric infarction, necrotizing enterocolitis and ulcerative colitis (Edelson *et al.* 1993, Kanda *et al.* 1996, Drapalo *et al.* 2008).

Most of the enteric pathogens damage the intestinal mucosa leading to oxidative stress that leads to an imbalance between oxidants and antioxidants at the cellular or individual level (Trevsian *et al.* 2001, Lykkesfeldt and Svendsen 2007). The tissue damage attributed by oxidative stress plays a key role in pathogenesis in enteric diseases of farm animals and dog (Ranjan *et al.* 2006, Panda *et al.* 2009, Kim *et al.* 2012, De *et al.* 2014). Bandyopadhyay *et al.* (2015) reported that *E. coli* diarrhea causes marked oxidative stress in young yak. Standard treatment of *E. coli* diarrhea is based on antimicrobial and fluid therapy. Such therapy can limit the multiplication of microorganism but it does not ameliorate the oxidative stress on host. Further, researchers reported that antibiotic therapy also induces oxidative stress on host and it may lead to antibiotic resistance (El-Halfawy and Valvano 2014). Therefore, supplementation of antioxidants could be a choice for better therapeutic management of *E. coli* enteritis and reduce the chance of antibiotic resistance. Antioxidant supplementation has been reported to prevent diarrhea in calves (Cummins and Brunner 1989, Sahinduran and Albay 2004). One such novel antioxidant is Coenzyme Q10 (CoQ10), a lipid-soluble molecule, which resides in the inner mitochondrial membrane where it acts as a mobile electron and proton transporter from electron transport chain complex I (NADH:

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ubiquinone reductase) and complex II (succinate: ubiquinone reductase) to complex III (ubiquinone cytochrome c oxidase). CoQ10 in its reduced form, ubiquinol, is recognized as an antioxidant and free radical scavenger, protecting membrane lipids, proteins and mitochondrial DNA against oxidative damage (Linnane *et al.* 2007). CoQ10 is used as an antioxidant in diseases like cancer, cardiac failure, bacterial periodontal disease and diabetes (Roffe *et al.* 2004, Adarsh *et al.* 2008, Prakash *et al.* 2010, Golbidi *et al.* 2011). However, the antioxidant effect of CoQ10 in neonatal *E. coli* calf diarrhea has not been reported till now. Therefore, present study was undertaken to examine the effect of CoQ10 on oxidative stress and intestinal fatty acid binding protein in calf diarrhea caused by *E. coli.*

MATERIALS AND METHODS

Selection of animals and diagnosis of E. coli *in fecal samples:* The present study was conducted in crossbred calves aged between 0–4 weeks of either sexes at Cattle and Buffalo Farm of the institute. A thorough clinical examination was performed for all the calves by expert clinician during the study. Diarrhea was diagnosed on the basis of clinical symptoms such as frequency of defecation (>3 times in a day), fecal consistency score (McLamb *et al.* 2013) and status of dehydration (Constable *et al.* 1998). The fecal samples from diarrheic calves were collected aseptically in sterile container (Sterile clinicol^{TM}, Himedia laboratories Pvt. Ltd., Mumbai, India) and transported on ice to laboratory for diagnosis of *E. coli.* Screening of *E. coli* in fecal samples was carried out by culture in specific bacteriological media and biochemical tests (Quinn *et al*. 2004).

Experimental design: Out of 87 fecal samples collected from diarrheic calves, 23 samples were found positive for *E. coli* based on cultural and biochemical tests. From these 23 *E. coli* positive diarrheic calves, 12 calves were selected and randomly divided into 2 equal groups (Group 2 and 3), each group containing of six numbers of *E. coli* positive diarrheic calves. Simultaneously, six healthy calves without any clinical signs of diarrhea were also included for the study and served as healthy control (Group 1). The unit of the study was calf. Healthy calves (Group 1) received placebo therapy which included sterile water twice daily for five days. Six diarrheic calves (Group 2) were treated with standard treatment (ST) consisting of Ceftriaxone @ 10 mg/kg bwt IM bid for 3 days, fluid (ringer lactate) bid IV based on dehydration and anti-inflammatory drug (Meloxicam @ 0.2 mg/kg bwt IM) when indicated; whereas, another six diarrheic calves (Group 3) received standard treatment as above along with coenzyme Q10 @ 5.0 mg/kg body weight orally once daily for five days. The experimental protocol was approved by the committee for the purpose of control and supervision of experiments for animals (No. 25/08/2016-CPCSEA), India.

Collection of blood samples: The blood samples (approximately 6.0 ml) were collected from each calf by venipuncture of jugular vein. Blood samples (2 ml) were collected in K_2 EDTA containing vials for hematology and 4.0 ml of blood samples collected in a sterile test tube without any anticoagulant for biochemical studies. After one hour of clotting, serum was separated by centrifugation at 3,000 rpm for 5 min and utilized for measurement of serum biochemistry, oxidative stress indices and intestinal fatty acid binding protein 2 (IFABP 2).

Haematology, serum biochemistry and clinical observations: For hematology, hemoglobin (Hb%), packed cell volume (PCV%) and total leukocyte count (TLC) were measured in whole blood as per standard protocol (Jain 1986). Spectrophotometric methods were employed to determine the levels of total protein (TP) and albumin (Verley 1980), blood urea nitrogen (BUN) (Wybenga *et al.* 1971) and creatinine (Frankel *et al.* 1970). Clinical recovery was assessed on the basis of fecal consistency score (McLamb *et al*. 2013) and status of dehydration (Constable *et al.* 1998).

Estimation of oxidative stress indices: Lipid peroxidation was measured by determining the plasma malondialdehyde (MDA) concentration by double heating method (Draper and Hadley 1990). The concentration of plasma MDA was calculated from the absorbance coefficient (1.56×105 cm^{-1} M^{-1}) of the thiobarbituric acid- malondialdehyde (TBA-MDA) complex. The nitrite and nitrate (NOx) in the plasma was measured by reduction of nitrate with activated copper-cadmium alloy and zinc sulfate followed by color development with Griess reagent (Sastry *et al.* 2002). The plasma Glutathione S-transferase (GST) activity assay was measured by using commercially available kit (EZAssay^{TM} GST Activity Estimation Kit as per the manufacturer's instruction.

Estimation of intestinal fatty acid binding protein 2 (IFABP2): The concentration of IFABP 2 in serum was measured using commercially available ELISA kit (IFABP 2, Cloud-clone corp*,* Houston, USA) as per the instruction given by manufacturer.All the parameters were measured before treatment (day 0) and thereafter on day 3 and 5 after initiation of treatment.

Statistical analysis: The data were analyzed by repeated measurement model with calf as subject and period as repeated measurement using statistical software package SAS (v 9.3). The one-way ANOVA was used to compare the treatments to hematology and oxidative stress indices tested. When an interaction was found, Tukey's post hoc test was used to determine statistical significance between the different treatment groups. A probability level (P) of 0.05 was selected as the statistical selection limit for all tests. Results were expressed as means ± SEM.

RESULTS AND DISCUSSION

On clinical examination, the affected calves exhibited the signs of high rise in temperature, diarrhea, severe dehydration, weakness and anorexia. Based on clinical signs, a total 87 fecal samples from diarrheic calves were collected for diagnosis of *E. coli.* Out of 87 fecal samples,

23 samples were found positive for *E. coli* based on growth of pink colonies and metallic sheen on MacConkey's agar and eosin methylene blue agar media, respectively. Further, biochemical tests revealed positive Indole and Methyle red tests; whereas, negative for Voges-Proskauer and Citrate utilization tests. The *E. coli* diarrhea in calves causes acute clinical symptoms characterized by anorexia, frequent defecation of loose feces, dehydration, dull and depression with sunken eye ball. In the present study, the mean values of fecal consistency score and dehydration (%) were significantly (P<0.05) increased in diarrheic calves (Gr. 2 and Gr. 3) as compared to healthy calves (Gr. 1). The findings of the present study were in accordance with previous workers (Bellamy and Acres 1979, Kumar *et al.* 2010, Wadhwa *et al.* 2006). The watery diarrhea associated with dehydration may be due to efflux of fluid into intestinal lumen induced by *E. coli* toxins as well as peristaltic movement. Before initiation (day 0) of treatment, the mean Hb, PCV and albumin, BUN and creatinine concentrations were significantly (P > 0.05) increased in diarrheic calves (Gr. 2 and 3) compared to healthy calves (Gr. 1). Marked hemoconcentration and rise of BUN and creatinine concentrations in diarrheic calves compared to healthy calves could be due to fluid loss from vascular compartment, hypovolemia and reduced renal flow and glomerular filteration rate leading to decreased excretion of urea and creatinine (Rajora and Pachauri 2000, Singh *et al*. 2014). Whereas, a significant (P< 0.05) increase in TLC in diarrheic calves in comparison to healthy calves might be due to normal body defense mechanism to control bacterial infection. The findings of the present study were in accordance with the previous workers (Malik *et al.* 2013, Singh *et al*. 2014).

The Hb, PCV and TLC level reduced significantly (P> 0.05) significantly on day 3 and 5 from day 0 value in *E. coli* diarrheic calves (Gr. 2 and Gr. 3) treated with either standard treatment (ST) or standard treatment plus CoQ10 (ST-CoQ10). The mean albumin and creatinine concentrations were unaffected throughout the study period (day 0 to day 5) in Gr. 2 diarrheic calves. However, ST-CoQ10 treatment significantly (P<0.05) reduced the albumin (on day 5), BUN and creatinine concentrations on day 3 and 5 in Gr. 3 diarrheic calves from pre-treatment value. When the values on day 3 and 5 in both the diarrheic groups (Gr. 2 and Gr. 3) were compared with healthy calves, it was found that TLC was significantly (P> 0.05) higher in diarrheic calves than healthy calves on day 3 (Table 1). The fecal consistency score and dehydration (%) significantly reduced on day 3 and 5 from day 0 value in *E. coli* diarrheic calves treated with either ST or ST- CoQ10 (Table 2). This can be correlated with effectiveness of therapy on restoration of hydration, reduction in frequency of defecation, correction of fluid balance and restoration of normal renal perfusion (Kumar *et al.* 2010, Leal *et al.* 2012). The reduction of TLC indicates effective antibacterial therapy. Similar findings were also recorded in other antimicrobial therapies in *E. coli* diarrhea in lamb (Hassan *et al*. 2013). The reduction of fecal consistency score and dehydration (%) in post treatment period in diarrheic calves either by ST or by ST-CoQ10 indicated clinical recovery of animal from diarrhea.

In the present study, pronounced (P<0.05) elevation of MDA, NOx and GST concentration in serum of diarrheic calves (Gr. 2 & 3) than healthy calves (Gr.1) before treatment (day 0) indicated that *E. coli* diarrhea leads to marked oxidative stress in calves. Oxidative stress associated with enteric diseases was reported in animals by previous workers (De *et al.* 2014, Ranjan *et al.* 2006, Bandyopadhyay *et al.* 2015). The oxidative stress linked with increased lipid peroxidation, MDA, nitric oxide and decreased catalase, superoxide dismutase and total antioxidant capacity were observed in acute calf diarrhea in cattle, buffalo and yak (Ranjan *et al.* 2006, Ahmed and Hassan 2007, Bandyopadhyay *et al*. 2015). The ST significantly reduced (P<0.05) the GST and NOx concentrations on day 3 from pretreatment value in diarrheic calves; however, MDA concentration remained persistently elevated (P>0.05) throughout the study period (day 0 to day 5) in diarrheic calves that received ST. When CoQ10 was incorporated in standard treatment of diarrheic calves (Gr. 3, ST-CoQ10), the concentrations of MDA, NOx and GST were significantly dropped on day 3 (P<0.05) and 5 (P<0.01) from pretreatment value (Table 1). Although the significant (P<0.05) reduction in MDA, NOx and GST activity was observed on day 3 and day 5 from day 0 value in both *E. coli* diarrheic calves treated with either standard treatment or Coenzyme Q10 plus standard treatment; however, such reduction was noted much earlier (day 3) in ST-CoQ10 treated group compared to ST treated group (day 5). It implicates that incorporation of Coenzyme Q10 in standard treatment ameliorated the oxidative stress much early. *In vitro* study demonstrated that the reduced form of CoQ10 prevents the oxidative stress by protecting membrane phospholipids and LDL from lipid peroxidation by increased antioxidant enzyme activity, and decreased the inflammatory marker like IL-6 in patients with atherosclerosis in humans (Cadenas *et al.* 1992, Lee *et al*. 2012). Moreover, authors also observed that CoQ10 supplementation reduced the MDA, GST and NOx concentrations in pulmonary contusion and cisplatin induced oxidative stress in experimental rat and mice model, respectively (Gokce *et al.* 2012, Sawicka *et al.* 2013).

In the present study, significant (P<0.05) increase of IFABP 2 concentration in diarrheic calves (Gr. 2 and Gr. 3) compared to healthy control (Gr. 1) indicates the injury of intestinal mucosa by the toxins of *E. coli*. During infection, *E. coli* generally attaches to intestinal mucosa, colonises there and produces heat stable and heat labile toxins. These pathologic changes result in ischemia and damage to epithelial cells, and consequently, lead to the impairment of the digestive and absorptive functions of epithelial and crypt cells and lead to diarrhea (Nagy and Fekete 1999). Niewold *et al.* (2004) reported that the concentration of plasma IFABP 2 in pigs and human coincides with increased

Table 1. Effect of standard treatment and standard treatment plus CoQ10 on hematology, serum biochemistry and oxidative stress indicators of *E. coli* associated diarrheic calves

Groups (n=6)	Days post treatment			Interaction‡		
	Day 0	Day 3	Day 5	T	D	T×D
Hb (g/dl)						
Hematology						
Gr. I	9.283±0.235$^{a, A}$	9.250±0.164$^{a, A}$	9.200±0.146$^{a, A}$	0.000	0.014	0.387
Gr. 2	10.850±0.418$^{a, B}$	9.966±0.352$^{b, A}$	9.750±0.348$^{c, A}$			
Gr.3	10.733±0.283 $^{a, B}$	10.033±0.185 $^{b, A}$	10.000±0.180 $^{b, A}$			
PCV(%)						
Gr. 1	33.83±1.167 $^{a, A}$	33.17±1.447 $^{a, A}$	34.00±0.365 $^{a, A}$	0.007	0.000	0.000
Gr. 2	42.00±0.816 $^{a, B}$	33.83±0.792 $^{b, A}$	33.17±1.108 $^{b, A}$			
Gr. 3	40.33±0.715 $^{a, B}$	33.33±1.229 $^{b, A}$	32.50±0.922 $^{b, A}$			
TLC (thousand cells/cu.mm)						
Gr. 1	8.333±0.365 $^{a, A}$	7.775±0.218 $^{a, A}$	8.191±0.247 $^{a, A}$	0.000	0.000	0.000
Gr. 2	13.425±0.256 $^{a, B}$	11.066±0.257$^{b, B}$	8.566±0.403$^{c, A}$			
Gr.3	13.566±0.125 $^{a, B}$	10.833±0.184 $^{b, B}$	8.858±0.292 $^{c, A}$			
Serum biochemistry						
Serum total protein (g/dL)						
Gr. 1	7.604±0.222 $^{a, A}$	7.739±0.175 $^{a, A}$	7.838±0.189 $^{a, A}$	0.568	0.456	0.366
Gr. 2	7.917±0.186 $^{a, A}$	7.833±0.177 $^{a, A}$	7.449±0.055 $^{a, A}$			
Gr. 3	7.614±0.156 $^{a, A}$	7.709±0.160 $^{a, A}$	7.475±0.180 $^{a, A}$			
Serum albumin (g/dL)						
Gr. 1	3.368±0.107 $^{a, A}$	3.464±0.127 $^{a, A}$	3.474±0.084 $^{a, A}$	0.493	0.042	0.119
Gr. 2	3.693±0.098$^{a, AB}$	3.543±0.098 $^{a, A}$	3.330±0.068 $^{a, A}$			
Gr. 3	3.555±0.067$^{a, AB}$	3.544±0.098 $^{ab, A}$	3.269±0.067 $^{b, A}$			
BUN (mg/dL)						
Gr. 1	17.563±1.6842 $^{a, A}$	16.950±0.899 $^{a, A}$	18.525±1.433 $^{a, A}$	0.329	0.000	0.009
Gr. 2	26.262±2.0016 $^{a, B}$	17.847±3.711$^{ab, A}$	15.196±1.244 $^{b, A}$			
Gr. 3	25.806±1.448 $^{a, B}$	16.059±0.438 $^{b, A}$	15.978±0.980$^{bc, A}$			
Creatinine (mg/dL)						
Gr. 1	0.875±0.262 $^{a, A}$	1.194±0.210 $^{a, A}$	1.202±0.094 $^{a, A}$	0.003	0.021	0.001
Gr. 2	1.736±0.155 $^{a, B}$	1.402±0.163 $^{a, A}$	1.472±0.192 $^{a, A}$			
Gr. 3	2.176±0.100 $^{a, B}$	1.319±0.104 $^{b, A}$	1.004±0.108$^{c, A}$			
Oxidative stress indicators						
MDA (nmol/ml)						
Gr.1	1.163±0.124 $^{a, A}$	0.961±0.142 $^{a, A}$	1.006±0.215 $^{a, A}$	0.000	0.000	0.000
Gr. 2	3.646±0.173 $^{a, B}$	3.154±0.235 $^{a, B}$	2.852±0.196 $^{a, B}$			
Gr. 3	3.624±0.123 $^{a, B}$	2.304±0.109$^{b, C}$	1.085±0.083 $^{c, A}$			
NOx (µmol/ml)						
Gr. 1	1.325±0.244 $^{a, A}$	1.267±0.150 $^{a, A}$	1.036±0.257 $^{a, A}$	0.000	0.000	0.000
Gr. 2	4.827±0.112 $^{a, B}$	3.620±0.084^{b,B}	3.620±0.084^{b,B}			
Gr. 3	5.226±0.217$^{a, C}$	2.693±0.049$^{b, C}$	1.514±0.277 $^{c, A}$			
GST activity (mol/ml/min)						
Gr. 1	0.337±0.0511 $^{a, A}$	0.325±0.0347 $^{a, A}$	0.266±0.0229 $^{a, A}$	0.000	0.000	0.000
Gr. 2	0.950±0.0695 $^{a, B}$	0.925±0.0849$^{a ,B}$	0.720±0.0653^{b,B}			
Gr. 3	1.045±0.0497 $^{a, B}$	0.629±0.0640$^{bc, C}$	0.479±0.0656$^{c, A}$			

Gr. 1, Six healthy calves received placebo treatment; Gr. 2, Six *E. coli* associated diarrheic calves treated with standard treatment (antibiotic, fluid and non-steroidal anti-inflammatory drug); Gr.3, Six *E. coli* associated diarrheic calves treated with standard treatment along with CoQ10. $^{abc/AB}$ Means (±standard error) bearing different superscripts in a row (a, b, c) or column (A, B) differ significantly (P<0.05); ‡Significant effects (p value) of treatment (T), period (P) or their interaction (T×P).

Table 2. Effect of standard treatment and standard treatment plus CoQ10 on dehydration (%) and fecal consistency score of *E. coli* associated diarrheic calves

Groups (n=6)	Days post treatment			Interaction‡		
	Day0	Day3	Day5	T	D	T×D
Dehydration (%)						
Gr. 1	< 5.000±0.000^{a,A}	<5.000±0.000^{a,A}	<5.000±0.000^{a,A}	0.000	0.000	0.000
Gr. 2	9.167±0.401^{a,B}	6.333±0.421^{b,A}	<5.000±0.000^{c,A}			
Gr. 3	8.667±0.333^{a,B}	5.500±0.562^{bc,A}	<5.000±0.000^{c,A}			
Faecal consistency score						
Gr. 1	0.33±0.211^{a,A}	0.33±0.211^{a,A}	0.50±0.224^{a,A}	0.000	0.000	0.000
Gr. 2	3.50±0.224^{a,B}	1.33±0.333^{b,A}	0.33±0.211^{c,A}			
Gr. 3	3.17±0.307^{a,B}	1.17±0.307^{bc,A}	0.50±0.224^{c,A}			

Gr. 1, Six healthy calves received placebo treatment; Gr. 2, Six *E. coli* associated diarrheic calves treated with standard treatment (antibiotic, fluid and non-steroidal anti-inflammatory drug); Gr, 3, Six *E. coli* associated diarrheic calves treated with standard treatment along with CoQ10; $^{abc/AB}$ Means (±standard error) bearing different superscripts in a row (a, b, c) or column (A, B) differ significantly (P<0.05); ‡Significant effects (P value) of treatment (T), period (P) or their interaction (T×P).

intestinal permeability resulting from ischemia. Further, they opinioned that IFABP 2 concentrations can be used as sensitive marker of mild damage to the intestinal mucosa in porcine model. Kanda *et al*. (1996) observed similar findings in animal experimentations. The standard therapy along with coenzyme Q10 reduced IFABP 2 much earlier as compared to Gr. 2 diarrheic calves (Fig 1). Although, no such literatures are available on the effect of coenzyme Q10 on IFABP 2; however, it has been reported that other antioxidant (like N-acetyl-cysteine, Lipoxin A4) reduces the plasma level of IFABP 2 in intestinal ischemia of rat and mice model (Khadaroo *et al*. 2014).

From the findings of the present study, it can be concluded that the coenzyme Q10 effectively reduced the oxidative stress indicators and IFABP 2 much earlier compared to standard treatment alone. The incorporation of CoQ10 in standard treatment will be helpful for early clinical recovery in *E. coli* associated calf diarrhea.

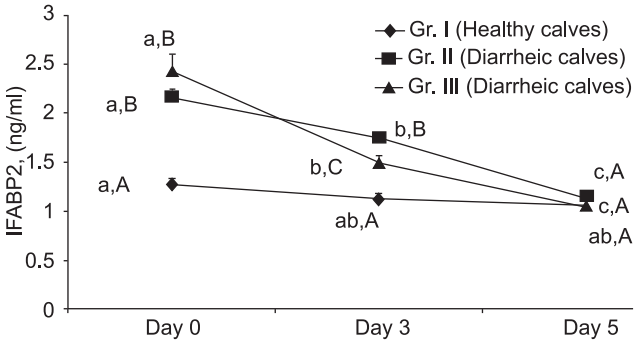

Fig. 1. Effect of standard treatment and standard treatment plus CoQ10 on concentration of intestinal fatty acid binding protein 2 (IFABP 2, ng/ml) in serum of *E. coli* associated diarrheic calves; Gr. 1, Six healthy calves received placebo treatment; Gr. 2, Six *E. coli* associated diarrheic calves treated with standard treatment (antibiotic, Fluid and NSAID); Gr.3, Six *E. coli* associated diarrheic calves treated with standard treatment along with CoQ10; $^{abc/ABC}$ Significant difference at P<0.05.

However, the therapeutic efficacy of CoQ10 involving large number of calves with *E. coli* and other enteric pathogens need further study to extract the full potential of the compounds with comprehensive results.

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