

*Full Length Research Paper*

# Trace elements changes in the appendix of rabbit infected with *Eimeria coecicola*

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The parasitic protozoa, *Eimeria coecicola*, causes intestinal coccidiosis in rabbits and, thereby, enormous economic losses in rabbit farms. This study aimed to investigate the trace elements changes in the appendix of infected rabbits with *E. coecicola*. Rabbits were orally infected with 50000 sporulated oocysts of *E. coecicola*, began to shed parasitic oocysts with their faeces on day 5 post-insemination (p.i.) and approximately 1.1 million oocysts were maximally shed on day 7 p.i. At maximal shedding, animals were euthanized and appendiceal samples were collected. The coccidial infection was accompanied with a decrease in weight of infected rabbits reaching 25% at day 7 p.i. Our results showed that the concentration of iron, boron, cadmium, cobalt, manganese and zinc was significantly increased due to *E. coecicola* infection. On the contrary, the concentration of sodium, potassium and calcium was not significantly changed. Also the number of goblet cells in the appendiceal villi was significantly decreased. It was shown that *E. coecicola* infection has induced an alteration in the level of some trace elements as well as goblet cells.

**Key words:** Trace elements, *Eimeria coecicola*, rabbits.

## INTRODUCTION

Coccidial infection remains one of the most economically important diseases of farm animals such as cattle, rabbits and poultry. The obligate intracellular protozoan parasite *Eimeria* sp. (Phylum: Apicomplexa) is a major causative agent of the disease (Pakandle, 2009). Rabbit coccidiosis is considered to be the most threatening factor affecting rabbit production (Peeters et al., 1984; Jithendran and Bhat, 1996) as it causes severe pathological changes to infected animals leading finally to huge economic losses in industrial rabbit farms (Baker, 2007; Taylor et al., 2007). Among the 800 known *Eimeria* species (Mehlhorn, 2001), there are eleven species which are specific for rabbits including *Eimeria coecicola* (Pakandl, 2009). Sporozoites of *E. coecicola* first invade the mucosa of the small intestine, especially the duodenum of the rabbits. Then, the parasites reach gut associated lymphoid tissue, including vermiform appendix, sacculus rotundus and Peyer's patches. Eventually, the appendix is the major

and final target site of *E. coecicola* (Pakandl et al., 2006). This target site has been previously investigated with respect to structural changes (Pakandle, 2009). Essential trace elements are micronutrients crucial for the host defense (Shankar and Prasad., 1998), including the development of inflammation (Milanino et al., 1993) and for the growth and virulence of many microorganisms (Beck et al., 1994; Krenn et al., 2005). Furthermore, the host may alter the micronutrient environment to deprive invading microorganisms of micronutrients that are essential for replication (Doherty et al., 2002). Non-essential trace elements, however, can compete and interact metabolically with essential trace elements, both in health and disease (Ilback et al., 2006). Metal-binding proteins, which include the family of acute-phase proteins, serve as carriers for essential trace elements. Consequently, there is a flux of trace elements between blood and other tissues during infection, including the tissues that are involved in the disease (Appendix in current study). Alterations in single essential and non-essential trace elements in blood have been described in bacterial, viral and parasitic infections (Frisk et al., 2007). Moreover, a number of studies have demonstrated trace

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**Table 1.** Oocyst output and weight change in rabbits at day 7 p.i. with *E. coecicola*.

Group	Oocyst shedding/g faeces	Weight on day 0 (g)	Weight on day 7 (g)	Weight change (%)
Non-infected rabbits	0	2000±98	2200±101	10
Infected rabbits	$11 \times 10^5 \pm 83666$	2100±103	1575±96	- 25

Values are means ± SD.

element changes in organs involved in the disease process (Ilback et al., 2004, 2007).

Goblet cells (GC) are glandular epithelial cells that coats the epithelium of digestive and respiratory tracts to protect them from chemical or mechanical damage, and to trap invading pathogens (De Paiva et al., 2011) so they have an important function in intestinal immune response against invading pathogens (Mcdole et al., 2012). Here, the appendiceal tissue of rabbits infected with *E. coecicola* was isolated and the induced changes in different trace elements as well as in the number of GC were investigated.

## MATERIALS AND METHODS

### Animals

New Zealand white rabbits, *Oryctolagus cuniculus* at an age of 7 to 9 weeks and a weight of 1.5 to 2.5 kg were obtained from the animal facilities of King Saud University. The animals were individually caged and were kept under constant conditions for at least 1 week before use. They are maintained as described by Licois et al. (1994). Their feces were examined daily during this week to assure the absence of any coccidial infection. The experiments were approved by the state authorities and followed the Saudi Arabian law on animal protection.

### Infection of rabbits

Animals were divided into 2 groups (8 animals each). The first group acted as the control non-infected group while the second group was the infected group with *E. coecicola* sporulated oocysts. *E. coecicola* used in this study is a pathogenic species of the small intestine. Oocysts were collected from faeces of rabbits naturally infected with *E. coecicola* and then surface sterilized with sodium hypochlorite and washed at least four times in a sterile saline solution prior to oral inoculation as described by Schito et al. (1996). These oocysts were used to inoculate rabbits by oral gavaging each rabbit receiving 50,000 sporulated oocysts of *E. coecicola* suspended in 1 ml sterile saline. Once every 24 h, fresh faecal pellets were collected and weighed for each rabbit and the bedding was changed to eliminate reinfection. Oocyst output was measured as previously described (Schito et al., 1996). Faecal pellets were suspended in 2.5% (wt/v) potassium dichromate and diluted in saturated sodium chloride for oocyst flotation. Oocysts were counted in a McMaster chamber and expressed as number of oocysts per gram of wet faeces.

### Sample collection

Eight infected and eight non-infected rabbits were killed and dissected on day 7 post-insemination (p.i.). Appendix was removed and cut into small pieces. Some were used to measure the

concentration of trace elements and the others were used for the GC scoring.

### Goblet cells

Small pieces of appendix were quickly removed, then fixed in 10% neutral buffered formalin. Following fixation, specimens were dehydrated, embedded in wax, and then sectioned to 5 µm thickness. Sections were then stained with periodic acid-Schiff's method to count the GC. For each animal, the number of GC in the appendix was counted on at least ten well-orientated villous-crypt units (VCU). Results were expressed as the mean number of GC per ten VCU (Allen et al., 1986).

### Assessment of trace elements in the intestinal appendix

Appendiceal samples were digested after drying according to Association of Analytical Communities (1995) methods. The levels of iron (Fe), boron (B), cadmium (Cd), cobalt (Co), manganese (Mn), zinc (Zn), sodium (Na), potassium (K) and calcium (Ca) in digests were determined with a Shimadzu atomic absorption spectrophotometer (air acetylene flame) using hollow cathode lamps operated at the recommended wavelength.

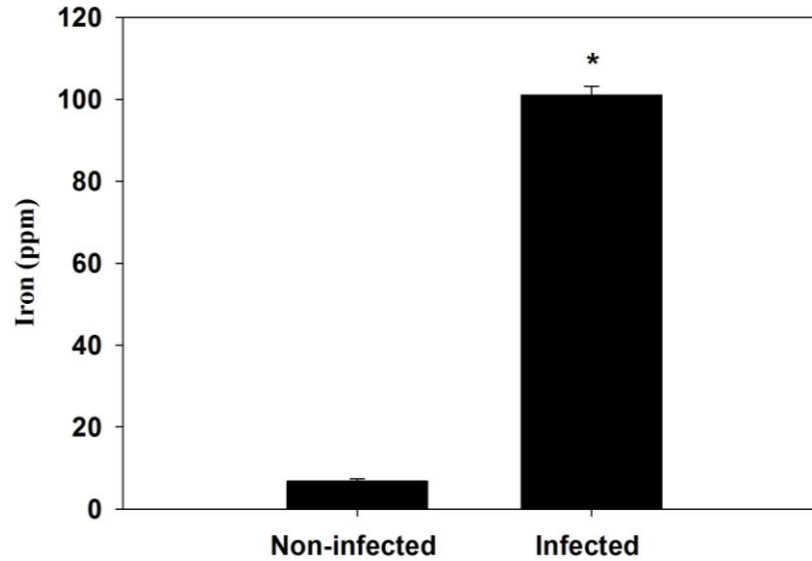
### Statistical analysis

Statistical analyses were performed using Student's *t*-test at  $P \leq 0.05$ .

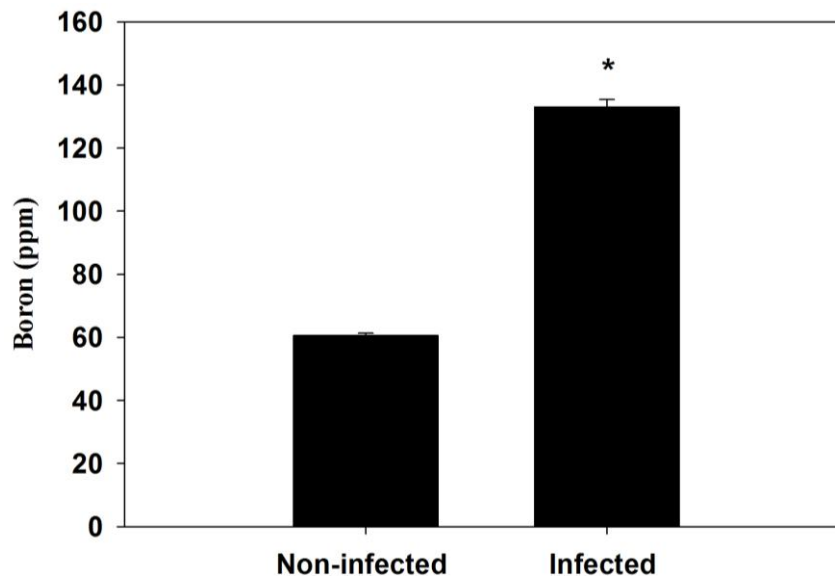
## RESULTS

Rabbits infected with *E. coecicola* began to express symptoms of coccidiosis that clearly appeared on day 7 p.i. where rabbits became weak and excrete watery mucoid diarrhea. The change in weight of animals was calculated whereas a reduction in weight of infected animals was obviously monitored and reached to about 25% weight loss at day 7 p.i. compared to increase in weight of control group by about 10% at day 7 p.i. (Table 1).

Changes in 9 trace elements were measured at day 7 p.i. In 6 of the measured elements, the changes were significant whereas the concentration of Fe has dramatically increased from  $6.75 \pm 0.6$  ppm in control to  $101.00 \pm 2.2$  ppm in infected rabbits (Figure 1). Also, B level was significantly increased ( $P < 0.001$ ) more than two folds (Figure 2). The concentration of Cd in the appendix was significantly elevated from  $3.18 \pm 0.6$  ppm to  $8.15 \pm 0.6$  ppm (Figure 3). The infection with *E. coecicola* was able to significantly increase the Co level to



**Figure 1.** Change in appendiceal Iron concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .

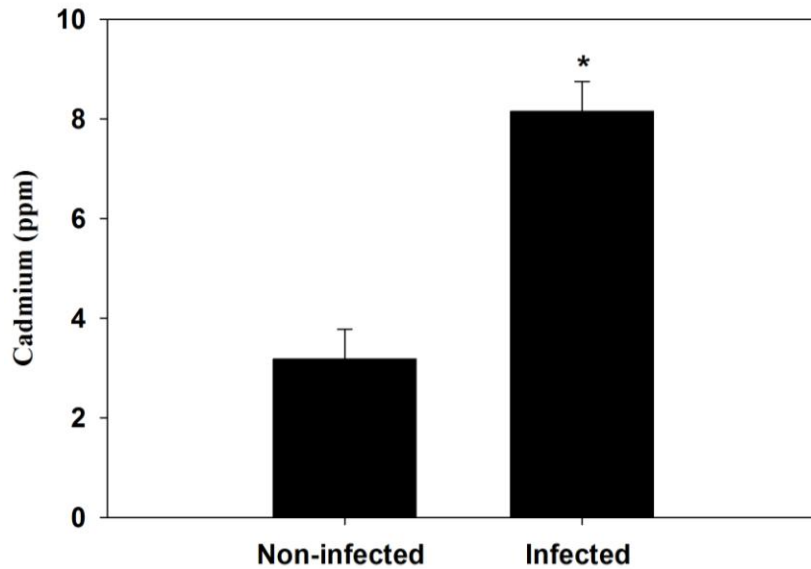


**Figure 2.** Change in appendiceal Boron concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .

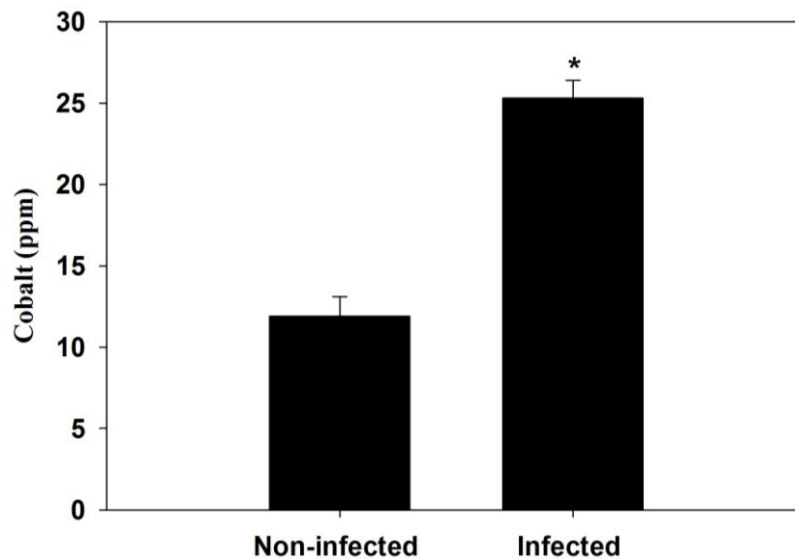
Approximately 50% (Figure 4). Moreover, Mn and Zn concentration has increased approximately about 3 fold in the infected rabbits (Figures 5 and 6). However, Ca, K and Na concentrations were not significantly changed (data not shown). Goblet cells are the major immunocompetent cells which act as a defensive barrier and are important to the normal physiological functions of the intestine. Our results based on PAS staining of appendix demonstrated that *E. coecicola* caused a significant decrease in the number of GC. This significant decrease reached about 35% (Figures 7 and 8).

## DISCUSSION

The final target site of *E. coecicola* is known to be the intestine, especially the appendix, though the early sporozoites have been also described to take an extra-intestinal route through mesenteric lymph nodes and the spleen before penetrating cells of the intestine (Renaux et al., 2001; Pakandl, 2009). Our data indicates at least that oral infection of the rabbit *Oryctolagus cuniculus* with 50,000 sporulated oocysts of *E. coecicola* results in alterations of the trace elements level. Here, we have



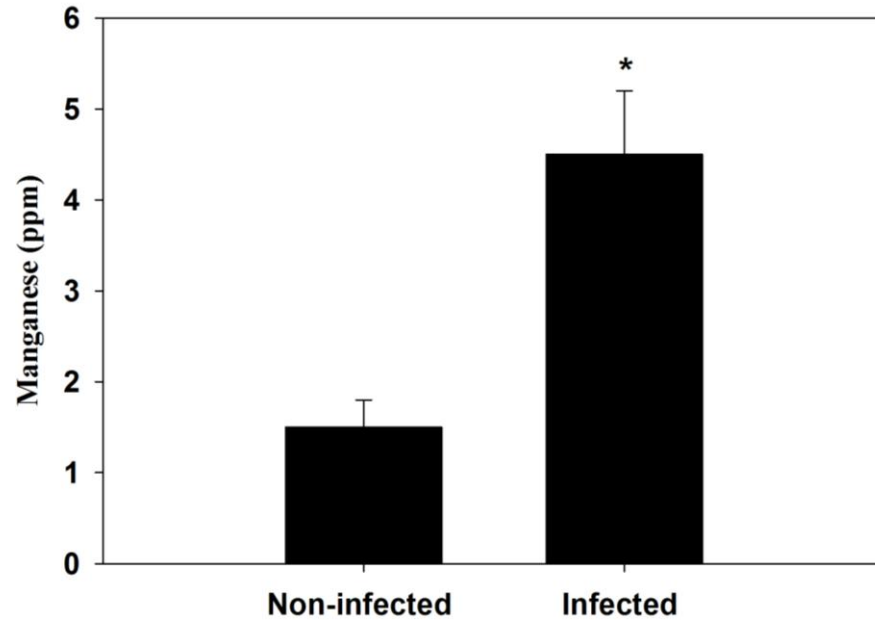
**Figure 3.** Change in appendiceal Cadmium concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .



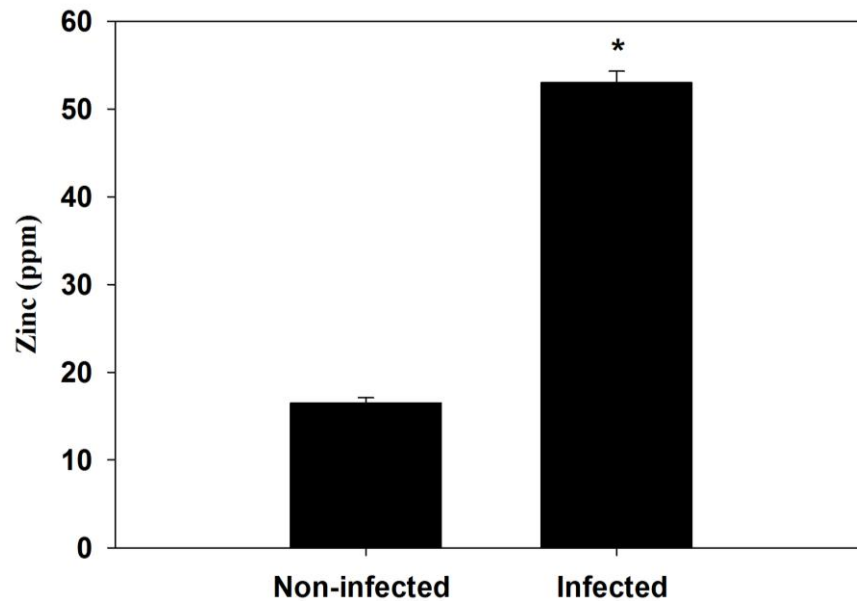
**Figure 4.** Change in appendiceal Cobalt concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .

elucidated that coccidial infection can alter the gastrointestinal uptake of both non-essential and essential elements. To meet the nutritional needs of the activated immune defense system, characteristic host metabolic responses in a generalized infection involve changes in protein, carbohydrate, lipid and trace element metabolism (Ilback et al., 2007). Alterations in essential and non-essential trace elements in blood have been described in bacterial, viral and parasitic infections (Frisk

et al., 2007). Moreover, a number of studies have demonstrated trace element changes in organs involved in the disease process (Ilback et al., 2004, 2007). As most infectious diseases are accompanied by a change in levels of several trace elements in the blood and tissue (Ilback et al., 2008) one aim of the present study was to identify infection associated changes of trace elements in the intestinal appendix during the course of *E. coecicola* infection in rabbits.



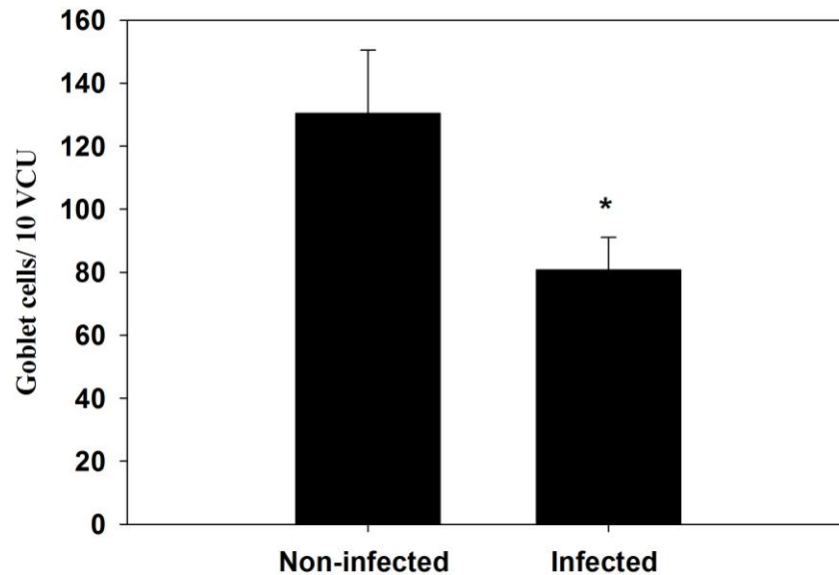
**Figure 5.** Change in appendiceal Manganese concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .



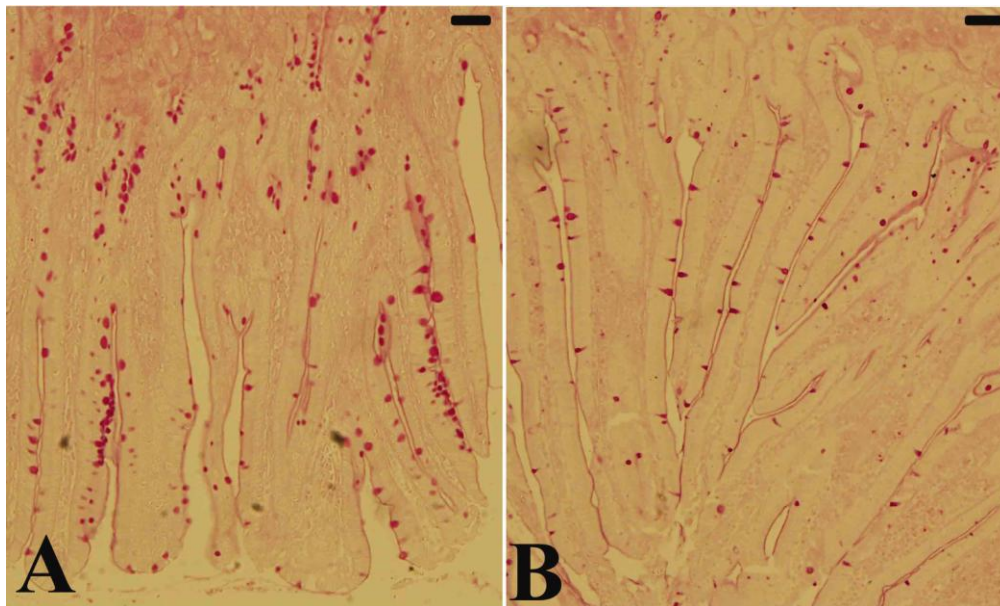
**Figure 6.** Change in appendiceal Zinc concentration after *E. coecicola* infection. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .

Oxidative stress has been reported in intestinal appendix of rabbits infected with *E. coecicola* (Dkhil et al., 2012) so the increase in iron concentration of appendiceal tissue may be a consequence of this process. However, non-essential trace elements like Cd can, if they are present in sufficient amounts, compete with essential elements like iron (Fe) (Goyer, 1997; Ilback

et al., 2006). Boron (B) is an essential trace element for animals and humans (Hunt and Idso, 1999). It plays a role in glycolysis, enzyme activity and respiratory burst (Hunt, 1998), mineral-mineral interactions (Devirian and Volpe, 2003), structural integrity of membranes (Fort et al., 1999), and cell-cell communication in bacteria (Chen et al., 2002). Supplemental B accelerates angiogenesis



**Figure 7.** Change in goblet cell number in appendix of rabbit infected with *E. coecicola* on day 7 p.i. Values are means  $\pm$  SD. \*: Significance at  $P \leq 0.05$ .



**Figure 8.** Changes in goblet cell numbers in rabbit appendix infected with *E. coecicola* on day 7 p.i. (A) Non-infected appendix (B) Infected appendix with decreased number of goblet cells. Sections are stained with periodic Schiff's stain. Bar=100  $\mu$ m.

and increases TNF $\alpha$  in cultured human fibroblasts (Benderdour et al., 1998), antibody concentrations in rats (Bai et al., 1997), and cytokines in pigs but down regulates porcine inflammatory responses (Armstrong and Spears, 2003). So the increased B concentration in current study may be considered as immune response strategy against the infection. Cobalt is an essential trace element and its biological activity is mainly confined to

the action of vitamin B12 coenzymes (Hughes, 1981) which play a significant role in the production of erythrocytes and the prevention of anaemia (Vellema et al., 1996). An excess of dietary Co in small doses increases the gain of body weight and decreases host mortality in *Ascaridia galli*-infected Hisex chickens (Gabrashanska et al., 2002). Manganese is a component of at least two metalloenzymes: Superoxide dismutase

and pyruvate carboxylase, which play a significant role in antioxidant protection and energy metabolism (Gillanders et al., 2008) so its increase is also a defensive mechanism utilized by the host towards oxidative stress induced by parasitic infection. As Zn supplementation lowered lipid peroxidation, it may have antioxidant activity. Since Zn is not redox active, it may not act directly as a scavenging antioxidant but instead may act as an indirect antioxidant by competing with pro-oxidant metals such as Fe and Cu for strategic binding sites (Beattie and Kwun, 2004; Ren et al., 2005; Watt et al., 2006). Goblet cells are glandular epithelial cells – found predominantly in the lining of the digestive and respiratory tracts – whose sole purpose is the secretion of mucus, a sticky, viscous substance composed of mucins, enzymes, and electrolytes suspended in water. It coats the epithelium of vulnerable structures to protect them from chemical or mechanical damage, and to trap invading pathogens (De Paiva et al., 2011). The numbers of large intestinal GCs (cecum and colon) gradually decreased (hypoplasia) in association with development of endogenous stages of parasite life cycle (Yunus et al., 2005). Also, the GC response in the small intestinal epithelium of C57BL/6 mice with *Eimeria vermiformis* infection on day 6p.i. was found to be significantly decreased due to infection (Linh et al., 2009). The decrease in number of GCs results directly or indirectly from infection of the intestinal crypts. *E. coecicola* parasites develop in the crypt region contains the multipotential stem cells (Cheng, 1974). GCs arise by mitosis from stem cells at the crypts (Metwaly et al., 2012).

In conclusion, the results of this study indicate that parasitic infection of rabbits with *E. coecicola* parasites induces a disturbed trace elements imbalance and a change in intestinal goblet cells in the intestinal appendix of infected rabbits.

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