CHANGES OF OXIDANT-ANTIOXIDANT PARAMETERS IN SMALL INTESTINES FROM RABBITS INFECTED WITH *E. INTESTINALIS* AND *E. MAGNA*

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Abstract: Rabbit coccidiosis is a very serious disease caused by protozoan parasites of the genus *Eimeria*, which increases the production rate of free radicals, especially reactive oxygen species. When the generation of free radicals exceeds the scavenging capacity of the body’s antioxidant system, the oxidant-antioxidant balance is broken, resulting in oxidative stress. This study was designed to investigate the effect on the oxidant-antioxidant status of rabbits infected with *E. intestinalis* and *E. magna*. To this end, eighteen 30-d-old weaned rabbits were randomly allocated into three groups as follows: the *E. intestinalis* infection group with $3 \times 10^7$ sporulated oocysts of *E. intestinalis*, the *E. magna* infection group with $20 \times 10^3$ sporulated oocysts of *E. magna*, and the uninfected control group. We measured the activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) and the contents of malondialdehyde (MDA) in rabbits’ small intestinal tissues (duodenum, jejunum and ileum) of the three groups on day 8. The results showed that CAT activity and MDA levels significantly increased, while the activities of SOD, GSH-Px and T-AOC decreased after *E. intestinalis* and *E. magna* infection. Besides, the jejunum and ileum were particularly damaged in the rabbits. It is concluded that the pathological oxidative stress occurs during the *E. intestinalis* and *E. magna* infection process and the body’s oxidant-antioxidant balance is disrupted.

Key Words: *E. magna*, *E. intestinalis*, oxidative stress, oxidant-antioxidant balance, rabbit.

INTRODUCTION

China is the highest rabbit producing country worldwide. Rabbit meat is regarded as a kind of meat with high protein, low fat and low cholesterol, which has high nutritional value (Dalle Zotte and Szendrő, 2011). However, coccidiosis is the most common parasitic infectious disease in rabbits, which can cause serious economic losses to the rabbit breeding industry by increasing mortality and reducing growth rates (Jing *et al.*, 2012; Li *et al.*, 2019). Coccidiosis is a serious problem in rabbit breeding, both in large commercial farms as well as small home farms and experimental farms, etc. (Balicka-Ramisz *et al.*, 2021). In clinical practice, rabbit coccidiosis is generally caused by a mixed infection of multiple *Eimeria* species (Hamid *et al.*, 2019), which can bring significant pathogenicity to the host, such as bloody stools, reduced feed conversion rate, slow growth rate and even death (Coudert *et al.*, 1993). Depending on the different developmental cycles of *Eimeria*, it can occur in the intestine or liver and spread rapidly (García-Rubio *et al.*, 2017). At present, there are 11-15 internationally recognised *Eimeria* species: *Eimeria stiedai* parasitises the liver, while the other species of *Eimeria* parasites different parts of the intestine (Eckert *et al.*, 1995; Li and Ooi, 2009).
Eimeria intestinalis is considered to be the highly pathogenic and highly immunogenic species, being fatal to rabbits and causing high mortality (Licois et al., 1990). Eimeria magna is recognised as a mildly pathogenic species, and E. magna infection usually does not result in death, even after inoculation with high oocyst numbers (Nilol, 1967; Licois et al., 1995). The infections of E. intestinalis and E. magna are chiefly caused by sporozoites. Sporozoites first invade the mucosa of the rabbit duodenum, with further development occurring in the jejunum and ileum (Shirley et al., 2005; Pakandl et al., 2006).

When the animal body is in a state of oxidative stress, it will lead to the excessive production of reactive oxygen species (ROS), or the body’s ability to eliminate ROS will be weakened, not only resulting in the destruction of the balance between oxidants and antioxidants, but causing tissue damage to the body (Burton and Jauniaux, 2011). Therefore, oxidative stress can be directly assessed by measuring ROS, and also be indirectly assessed by the associated damage to lipids, proteins and nucleic acids caused by ROS overproduction (Dinstel et al., 2013). At the same time, one of the mechanisms that protect the host cells against excess free radicals is the enzymatic antioxidant defence, which includes SOD, catalase (CAT), glutathione peroxidase (GSH-Px) and total antioxidant capacity (T-AOC) (Hirrlinger et al., 1999). Among them, SOD seems to be the first line of defence against ROS, while GSH-Px and CAT are both main oxidoreductases that can decompose hydrogen peroxide (Cecerska-Heryć et al., 2021). T-AOC refers to the total antioxidant level of various antioxidant substances and antioxidant enzymes, such as antioxidant enzymes, vitamin C or vitamin E (Saita et al., 2014), which can scavenge ROS to protect the body from oxidative stress damage, so it is used to evaluate the antioxidant system as an important comprehensive indicator (Hussain et al., 2012). Moreover, as a by-product of lipid peroxidation, MDA can be used for evaluating lipid peroxidation, in order to indirectly reflect the degree of damage to the body (Bahrami et al., 2016; Lin et al., 2019). According to the recent literature, the altered activities of some antioxidant substances and antioxidant enzymes in parasitic diseases and coccidiosis have been reported (Matés et al., 1999; Georgieva et al., 2006; Wang et al., 2020), but there was lack of information on the changes of oxidative stress-related parameters in the intestinal tissues of rabbits infected with E. intestinalis and E. magna. Therefore, the present study focused on measuring MDA levels and antioxidant enzyme activities of SOD, CAT, GSH-Px and T-AOC in the intestinal tissues of rabbits infected with E. intestinalis and E. magna. This study explored the relationship between oxidative damage and coccidiosis, which provided an important scientific basis for the prevention and treatment of coccidiosis.

**MATERIALS AND METHODS**

**Experimental animals**

A total of eighteen 30-d-old healthy rabbits (weight=1021±40.42 g) were purchased from Ganzhou Animal Husbandry Research Institute (Ganzhou, Jiangxi, China). Before the formal experiment, the rabbits had access to feed and water freely in the disinfection and coccidia-free laboratory (Animal Anatomy Laboratory of Jiangxi Agricultural University), and then the oocysts of coccidia were examined in rabbits by saturated brine flotation every day until no oocysts were observed up to two weeks to meet the experimental requirements (Shi et al., 2016).

**Oocysts**

Oocysts were donated by the College of Veterinary Medicine, South China Agricultural University. The oocysts were preserved in 2.5% potassium dichromate solution at 4°C after proliferation, collection and sporulation. Before inoculation, the oocysts were washed with normal saline solution to remove the potassium dichromate by repeated dilution and centrifugation.

**Sample preparation**

The rabbits (n=18) that met the requirements were randomly divided into three groups with six rabbits per group: the first group infected with 3×10^3 E. intestinalis oocysts (Coudert et al., 1993), the second group infected with 20×10^3 E. magna oocysts (Tao et al., 2017), and the third group injected with the same volume of phosphate buffered saline as the first two groups, which was regarded as the control group. After the 8th day of infection (Yuan et al., 2021), all
three groups of rabbits were euthanised and the abdominal cavity was then dissected to quickly collect fresh small intestinal tissues (including duodenum, jejunum and ileum). Subsequently, 200 mg of each intestinal tissue was added to a sterile tube containing 1.8 mL normal saline and ground in a tissue grinder (Wuhan Servicebio Technology Co., Ltd), then centrifuged at 2000 rpm for 10 min, and the supernatant was collected and stored in a refrigerator at −20°C for antioxidant index detection. The above remaining fresh tissue samples could be rapidly frozen in liquid nitrogen and stored in a refrigerator at −80°C (Thermo Fisher Scientific, Waltham, MA, USA). All procedures in this experiment were carried out in accordance with the Ethical Guidelines on Animal Experiments and approved by the Animal Testing Welfare Ethics Committee of Jiangxi Agricultural University (approval number: JXAULL-2020-34).

Detection of oxidant-antioxidant parameters

In this experiment, the protein concentrations of small intestinal tissues (duodenum, jejunum and ileum) from three groups were detected using the bicinchoninic acid (BCA) protein concentration assay kit (Beyotime Biotechnology Co., Ltd, Shanghai, China). The five oxidant/antioxidant markers, including superoxide dismutase (Cat. No. A001-3, SOD), catalase (Cat. No. A007-1-1, CAT), glutathione peroxidase (Cat. No. A005, GSH-Px), malondialdehyde (Cat. No. A003-1, MDA), and total antioxidant capacity (Cat. No. A015-1, T-AOC) were strictly tested according to the manufacturer’s kit instructions (Nanjing Jiancheng Bioengineering Institute Inc., Nanjing, Jiangsu, China). In addition, each sample was repeated three times and the absorbances of SOD, CAT, GSH-Px, T-AOC and MDA were measured at 450, 405, 412, 520 and 532 nm using a microplate meter (Molecular Devices Co., Ltd, Shanghai, China).

Statistical analysis

All experimental data were expressed as means±standard errors, and one-way analysis of variance was performed on the data to determine the differences between groups, using SPSS 22.0 software (Chicago, IL, USA). Difference was considered significant if \( P<0.05 \). Lastly, the data were input into Graph Prism 8.0 software (GraphPad, San Diego, CA, USA) to form a corresponding histogram.

RESULTS

First of all, according to the assessments of the antioxidant enzyme activity and lipid peroxidation of small intestinal tissues, all of the oxidative stress-related parameters were within the normal range in the control group.

In the duodenum homogenates, compared with the control group, significant increases in MDA levels and CAT activity were observed in the rabbits infected with \( E. \) intestinalis and \( E. \) magna \( (P<0.01) \); the SOD activity was significantly lower than the control group \( (P<0.01) \), but the T-AOC activity was not significantly different \( (P>0.05) \). A significant reduction in GSH-Px activity of the \( E. \) intestinalis infection group was noticed as opposed to the control group \( (P<0.01) \), while the activity of GSH-Px was not found significant in the rabbits infected with \( E. \) magna \( (P>0.05) \) (Figure 1).

![Figure 1](image.png)

Figure 1: Comparative analysis on oxidant-antioxidant parameters in the duodenum respect to the control. *\( P<0.05 \); **\( P<0.01 \). ■ Control; ■ \( E. \) intestinalis infection; ■ \( E. \) magna infection.
Changes in oxidative stress-related parameters in the jejunum were observed in the rabbits on the 8th day after *E. intestinalis* and *E. magna* infection. Compared with the control group, the enzyme activities of SOD, GSH-Px and T-AOC were significantly decreased in both the *E. intestinalis* infection group and the *E. magna* infection group ($P<0.01$). However, the MDA contents and CAT activity were significantly higher in both infected groups than those of the control group ($P<0.01$) (Figure 2).

In the ileum homogenates, a highly significant increase in CAT occurred in the group infected with *E. intestinalis* ($P<0.01$), SOD and GSH-Px were obviously decreased, but T-AOC was reduced ($P<0.01$), while there was no significant difference in the contents of MDA compared with the control group ($P>0.05$). The significant increases in average MDA level and CAT activity were observed in the rabbits infected with *E. magna* compared to the control group ($P<0.01$). In contrast, the changes in SOD, GSH-Px and T-AOC activities were not significant on day 8 after *E. magna* infection ($P>0.05$) (Figure 3).

**DISCUSSION**

*Eimeria intestinalis* and *Eimeria magna* are pathogenic because they multiply in the intestine, severely damaging the integrity of the intestinal epithelial cells and intestinal villi, and resulting in the increase of toxic substances and the invasion of external bacteria or viruses (Pakandl et al., 2001). Meanwhile, *Eimeria* infection can cause a series of physiological and biochemical reactions in rabbits, leading to an imbalance of oxidation/antioxidant system and oxidative stress (Allen, 1997). Some related strategies controlling coccidiosis have been reported, including anticoccidial drugs and vaccination. Nevertheless, both methods had limitations and risks of drug residue, drug resistance and strain variation (Jang et al., 2010; Giannenas et al., 2012). These problems indicate that there is an urgent need to explore the correlation between *Eimeria* infection and the oxidation-antioxidant balance, in order to find new antioxidants and immunoprophylactic drugs to control coccidiosis in rabbits (Dalloul and Lillehoj, 2005). Therefore, the changes of oxidant-antioxidant parameters were investigated in the rabbits experimentally infected with *E. intestinalis* and *E. magna*.

SOD, CAT and GSH-Px are important enzymes involved in endogenous antioxidant defence against ROS, and these three enzymes can form mutually protective defence groups in the antioxidant defence system (He et al., 2017). SOD catalyses the superoxide (O$_2^−$) radical to form either ordinary molecular oxygen (O$_2$) or hydrogen peroxide (H$_2$O$_2$) (Fattman et al., 2003), which can protect CAT and peroxidase from inactivation and protect cells and tissues from damage. CAT and GSH-Px do not directly scavenge free radicals, but can catalyse the formation of H$_2$O from H$_2$O$_2$ and protect SOD (Cecerska-Heryć et al., 2021). In the present study, the activities of SOD and GSH in the small intestinal tissues of rabbits infected with *E. intestinalis* and *E. magna* showed a downward trend, while the CAT activity significantly increased. The change trend of enzyme activity in the *E. intestinalis* infection group was larger than that in the *E. magna* infection group. We believe that the increase in CAT activity may be the compensatory mechanism in

![Figure 2](image-url)  
**Figure 2:** Comparative analysis on oxidant-antioxidant parameters in the jejunum respect to the control. *$P<0.05$; **$P<0.01$. □ Control; □ *E. intestinalis* infection; □ *E. magna* infection.
the rabbits infected with coccidia. Some studies hypothesised that changes in SOD, CAT and GSH-Px activity during coccidiosis infection may be due to the body’s defence system being overwhelmed by excessive formation of reactive compounds, which indicated oxidative stress (Çam et al., 2008; Abdel-Haleem et al., 2017). Besides, Mengistu et al. (2021) reported that SOD and MDA in serum of chickens infected with Eimeria Tenella was significantly reduced and CAT activity increased, compared with the control group. Xiao et al. (2018) found that the activities of SOD and GSH-Px in chicken duodenum decreased after lipopolysaccharide (LPS) induction.

Lipid peroxidation is an important mechanism of cell membrane destruction. MDA is caused by lipid peroxidation of polyunsaturated fatty acids, and is used as a biomarker of oxidative stress (Romero et al. 1998). MDA can interact with amino groups of protein to form intermolecular cross-linking, which inactivates membrane-binding enzymes and receptors (Sasani et al., 2018). In addition, MDA not only reflects the speed and intensity of lipid peroxidation, but also indirectly reflects the degree of tissue peroxidation damage, so it can be used as one of the important parameters for potential antioxidant capacity (Gaschler and Stockwell, 2017). According to a previous study, Abdel-Haleem et al. (2017) indicated that the increase in MDA and NO in the rabbit ileum were due to a severe inflammatory response caused by oxidative damage from E. intestinalis infection, which was consistent with our study. Also, Dkhil et al. (2012) found that CAT activity and MDA contents significantly increased in rabbit appendices infected with Eimeria coeicola, which indicated that Eimeria coeicola infection caused tissue damage and oxidative stress. In present study, the increased MDA content in the intestinal tissues of rabbits infected with E. intestinalis and E. magna could be attributed to the increased ROS production, leading to lipid peroxidation.

Moreover, in order to protect cells and organisms from oxidative stress damage caused by reactive oxygen free radicals, the T-AOC can be used to evaluate the antioxidant capacity of bioactive substances (Hussain et al., 2012). Our data showed that the T-AOC activity in the jejunum and ileum of rabbits infected with E. intestinalis and E. magna was significantly lower than that of the control group, while there was no significant difference in the T-AOC of duodenum between the two groups. This may be due to the fact that the duodenum is not a specific site for the parasitic proliferation of E. intestinalis and E. magna, and the degree of damage to the duodenum during coccidia infection is relatively weak. At the same time, Shi et al. (2020) evaluated the changes in the antioxidant status of lambs under heat stress, and the decrease of T-AOC index indicated that heat stress damaged the antioxidant defence system of lambs. In the LPS-induced colitis experiment, Zhou et al. (2018) found that SOD and T-AOC decreased and MDA content increased, which indicated the occurrence of inflammatory damage and oxidative stress. Obviously, the present results are consistent with those reports.

**CONCLUSIONS**

In the E. intestinalis-infected and E. magna-infected rabbit models, the changes in oxidant-antioxidant parameters would probably be caused by the intestinal injury or excessive free radicals produced, ultimately leading to oxidative stress. At the same time, the changes in these indicators also showed that the rabbit body performed a defensive...
role to reduce the injury caused by coccidia infection. Here, both *E. magna* and *E. intestinalis* were parasitic in the small intestine, but the damage of duodenum, jejunum and ileum and the intensity of oxidative stress were different in rabbits infected with these two coccidia. The present results will provide an important reference basis for further exploring the pathogenic mechanism of rabbits during *E. magna* and *E. intestinalis* infection.

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**REFERENCES**


