Efficacy of *Commiphora molmol* against hepatic coccidiosis (*Eimeria stiedae*) in the domestic rabbit

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Abstract

This study investigated the effect of oleo-gum-resin from *Commiphora molmol* Engler (Family: Burseraceae), commonly known as myrrh and as the commercial extract Mirazid, as a treatment for hepatic coccidiosis induced by the parasite *Eimeria stiedae* in domestic rabbits. Rabbits (*Oryctolagus domesticus* L.) were infected with sporulated parasite oocysts and subjected to treatment regimens of crude-myrrh suspension or the oleo-resin extract, Mirazid, each administered at 500 mg/kg rabbit body weight. Treatment of the infected rabbits resulted in significant reduction of mean oocyst numbers in the faeces of crude-treated rabbits (52.38%) and Mirazid-treated rabbits (90.90%), as compared to the untreated infected rabbits at Day 21 post-infection (pi). At Day 28 pi, no oocysts were observed in the faeces of treated rabbits, and both treatments resulted in significant recovery from hepatic coccidiosis, as evidenced by normal levels of liver enzymes (alanine aminotransferase, aspartate aminotransferase, gamma glutamyl transferase, alkaline phosphatase and bilirubin), total protein and hemoglobin levels returned to normal in response to the treatments. The results also indicate that Mirazid was more effective than the crude myrrh, likely due to a higher content of the active ingredients. The results of this study indicate that myrrh extracts, such as Mirazid, are promising sources for novel effective anti-coccidial drugs that are safe for the animal and the environment. These extracts are recommended for use in clinical practices.

Key words: Hepatic coccidiosis, *Commiphora molmol*, biochemical analysis.

Introduction

Coccidiosis remains a serious problem on rabbit farms, causing high mortality with significant economic losses worldwide 1-3. Coccidiosis is caused by coccidian parasites of the genus *Eimeria*. Most of the current anti-coccidial drugs show low efficacy and cause deleterious side effects 4. The extensive use of chemical anti-coccidial drugs in controlling this disease has led to the development of drug-resistant parasites. Parasite resistance and the side effects of some of the anti-coccidial drugs will have serious consequences for future disease control 5-9. Therefore, to combat this disease there is a pressing need to identify new effective drugs that are safe for the animals and the environment.

Many studies have focused on the search for novel, safe, and effective anti-coccidial drugs from natural medicinal plants or herbs. Myrrh (Mirazid) is an oleo-gum-resin obtained from the stems of the medicinal herb *Commiphora molmol* (Family: Burseraceae), a small tree found naturally in the southern Arabian Peninsula, Yemen and Somalia. It is one of the oldest known traditional medicinal and food plants used by the ancient Egyptians and Arabs 11. *C. molmol* was approved by the United States Food and Drug Administration (FDA) for food use (Code of Federal Registration 21 CFR 172.510), and was given the generally recognized as safe (GRAS) status as a flavor ingredient (No. 2765) by the Flavor Extract Manufacturers Association (FEMA) 12,13. The Council of Europe (1981) included myrrh in the list of plants and parts acceptable for use in foods 14. Myrrh contains volatile oil (7-17%), resin (25-40%), gum (57-61%), and impurities (3-4%). The present work studied the anti-coccidial action of myrrh gum and Mirazid in experimentally-infected rabbits (*Oryctolagus domesticus* L.). Mirazid, the oleo-resin extract from myrrh, has a broad range of bioactivities, including anti-inflammatories 15,16, anti-cancer 17, anti-estrogenic 18,19, anti-ulcer 20 and anti-diabetic 21, for the treatment of many disease conditions. It is well tolerated with mild or no side effects and has been shown harmless toward many organs and biological components, such as liver, kidney, the hematopoietic system, chromosomes, and the foetal skeleton, despite repeated doses over a relatively long period 22,23. Moreover, myrrh extracts have been reported as a new safe and effective drug against the bilharziasis worms, *Schistosoma mansoni* and *S. haematobium* 24-26. In previous studies myrrh has been used as molluscicides, cercaricides 35,37,41-43 and insecticides 44. Myrrh has also been effective in the treatment of fascioliasis caused by *Fasciola* worms 50-54. Myrrh shows high efficacy against many helminthic diseases like *Dicrocoelium dendriticum* in humans and animals 55,56 and *Heterophyes* spp. 57,58. It also has cestodicidal activity 59 and is effective against many intestinal nematodes 60 and the tick *Argas persicus* 61. The use of myrrh has been reported since antiquity 62. The genus *Commiphora* is composed of more than 200 species, and has been exploited as a natural drug to treat pain, skin infections, inflammatory conditions, diarrhea and periodontal diseases. In more recent history, products derived from *C. myrrha* and various other species of *Commiphora* have been recognized to...
have significant antiseptic, anesthetic and antitumor properties. Traditional practice and evidence-based research suggest that these properties are directly attributable to terpenoids, especially furano-sesquiterpenes, the active compounds present in myrrh essential oil. Recently, studies have focused on applying clinical trial methodologies to validate use as anti-neoplastic and antiparasitic agents and in healing wounds. Therefore, this study was undertaken to determine the efficacy of myrrh for the treatment of coccidiosis.

Materials and Methods

Rabbit infection with *Eimeria stiedae* oocysts: *Eimeria stiedae* oocysts were collected from liver nodules or gallbladder lumens of naturally-infected rabbits. Oocysts were washed and concentrated by the flotation method. The sporulated oocysts were stored in 2.5% potassium dichromate at 4°C. Rabbits were inoculated with a dose of 1×10⁷ oocysts per rabbit via gastric inoculation.

Myrrh preparations: Crude myrrh (oleo-gum-resin) and Mirazid (the oleo-resin extract from myrrh) used in this study were produced by Pharco Pharmaceutical Company (Alexandria, Egypt). Myrrh contains a resin (myrrhin, 23-40%), a volatile oil (myrrhol, 7-17%), gum (2-8%) and bitter principle (40-60%), whereas Mirazid consists of 8 parts of resin and 3.5 parts of volatile oils. The Mirazid emulsion was prepared using Cremophore-EL as an emulsifying agent. Each rabbit was given a dosage of 500 mg/kg orally every morning after an overnight fast 1 hour before feeding for 14 consecutive days, beginning 14 days after inoculation.

Rabbits: The study used a total of 80 male New Zealand white rabbits aged 4-4.5 months, weighing 1200-2000 g, and free from coccidial infection. Faecal samples of these rabbits were determined free of oocysts for 3 successive days by use of the floating technique and counted using a haemocytometer on Days 1, 7, 14, 21, and 28 pi. The uninfected groups (G1, G3, G4, G6 and G7) remained healthy throughout the experiments without apparent changes in behaviour. In the infected groups (G2, G5, and G8), faecal parasite oocysts were observed on Day 14 pi. All three infected groups showed general symptoms characteristic for coccidiosis: reduction of food intake, anorexia, diarrhea with mucous in faeces, rough hair coat, hair loss and bloating. These symptoms were undertaken to determine the efficacy of myrrh for the treatment of coccidiosis.

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Body and liver weights: Rabbits were weighed at the beginning of the experiment and on Days 7, 14, 21, and 28 pi. Livers were taken from euthanized rabbits on Days 14 and 28 pi and relative liver weights were determined according to Gomez-Bautista et al.

Experiment design and rabbit groups examined: Treatment | Groups
--- | ---
Infected untreated control | G1
Infected untreated control | G2
Infected and given distilled water | G3
Infected and treated with *C. molmol* suspension | G4
Infected and treated with *C. molmol* suspension | G5
Infected and given emulsifying agent | G6
Infected and treated with Mirazid emulsion | G7
Infected and treated with Mirazid emulsion | G8

Blood sampling and serum analysis: Two blood samples were collected from ear veins on Days 1, 7, 14, 21 and 28 pi. Approximately 1 ml of blood was collected on depot salt of ethylene diamine tetra-acetate (EDTA) and used to analyze hemoglobin levels. Approximately 2 ml of blood were collected in an empty centrifuge tube and incubated in a sloped position to coagulate at room temperature prior to centrifugation at 1500 rpm (Hettich Zentrifugen, E13A20) for 10 min to separate sera. The separated serum was transferred to clean dry vials and used for determination of liver function enzymes using Reflotron kits (Roche Co., Germany). The following enzymes were measured: aspartate amino transferase (AST), alanine amino transferase (ALT), gamma glutamyl transferase (GGT), alkaline phosphatase (ALP) and bilirubin (Bil). Blood haemoglobin (Hb) was measured to test for anemia. Total protein (Tp) was also measured in serum using a UV/visible spectrophotometer (M501).

Statistical analyses: Data were analyzed using the SPSS package program (SPSS 8.0, 15.0 for Windows). Data were represented as arithmetic means with the standard error. One-way analysis of variance (ANOVA) and t-tests were performed to demonstrate differences between groups.

Results

The uninfected groups (G1, G3, G4, G6 and G7) remained healthy throughout the experiments without apparent changes in behaviour. In the infected groups (G2, G5, and G8), faecal parasite oocysts were observed on Day 14 pi. All three infected groups showed general symptoms characteristic for coccidiosis: reduction of food intake, anorexia, diarrhea with mucous in faeces, rough hair coat, hair loss and bloating. These symptoms

Table 1. Mean (Mean ± SE) of rabbits weight in all animals groups.

<table>
<thead>
<tr>
<th>Day</th>
<th>Groups</th>
<th>Group G1</th>
<th>Group G2</th>
<th>Group G3</th>
<th>Group G4</th>
<th>Group G5</th>
<th>Group G6</th>
<th>Group G7</th>
<th>Group G8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day one</td>
<td></td>
<td>1480±0.01</td>
<td>1640±0.08</td>
<td>1450±0.01</td>
<td>1470±0.01</td>
<td>1500±0.02</td>
<td>1460±0.02</td>
<td>1470±0.02</td>
<td>1500±0.02</td>
</tr>
<tr>
<td>7 Days After</td>
<td></td>
<td>1450±0.02</td>
<td>1450±0.04</td>
<td>1420±0.01</td>
<td>1450±0.02</td>
<td>1370±0.01</td>
<td>1420±0.01</td>
<td>1470±0.01</td>
<td>1370±0.02</td>
</tr>
<tr>
<td>inoculation</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>14 Days After</td>
<td></td>
<td>1480±0.01</td>
<td>1380±0.05</td>
<td>1450±0.01</td>
<td>1470±0.01</td>
<td>1120±0.02</td>
<td>1460±0.02</td>
<td>1470±0.02</td>
<td>1140±0.04</td>
</tr>
<tr>
<td>inoculation</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>21 Days After</td>
<td></td>
<td>1440±0.02</td>
<td>1120±0.04</td>
<td>1380±0.02</td>
<td>1500±0.03</td>
<td>1300±0.02</td>
<td>1400±0.03</td>
<td>1480±0.02</td>
<td>1180±0.04</td>
</tr>
<tr>
<td>inoculation</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>28 Days After</td>
<td></td>
<td>1480±0.02</td>
<td>1010±0.01</td>
<td>1640±0.19</td>
<td>1600±48.7</td>
<td>1371±0.03</td>
<td>1570±0.07</td>
<td>1542.8±36.8</td>
<td>1250±0.04</td>
</tr>
</tbody>
</table>

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appeared on Day 10 pi when the rabbits became emaciated. After treatment of the infected groups (G5 and G8) with the myrrh preparations, a remarkable improvement (increased food intake and weight gain, and ceased diarrhea, hair loss and bloating) was observed.

Table 1 and Fig. 1 summarize the body weight measurements for the different rabbit groups. There was a significant loss in body weight in the infected untreated control group (G2) throughout the course of the experiment. In the infected and treated groups (G5 and G8), body weights decreased after inoculation to 1120 g and 1140 g, respectively, at Day 14 pi. However, the G5 and G8 rabbits regained weight gradually after treatment to 1300 g and 1180 g, respectively, on Day 28 pi. The body weights of G5 and G8 rabbits were significantly higher than in G2 and closer to the body weights of rabbits in the uninfected untreated control group (G1) by Day 28 pi, and there was no significant difference in body weights between the crude suspension-treated G5 rabbits and the Mirazid-emulsion-treated G8 rabbits (P ≤ 0.10).

The mean oocyst counts and standard errors (SE) for all three infected rabbit groups on Days 14, 21, and 28 pi are represented in Table 2 and Fig. 2. Oocysts, as expected, were observed in the faeces on Day 14 pi in all three infected groups (G2, G5, and G8) with a mean of 30,000 (SE ± 4944.1), 30,002 (SE ± 7600.2), 39,000 (SE ± 8491.8) oocysts per gram (opg), respectively. Oocyst loads were reduced in G5 by 52.38% and in G8 by 90.90% on Day 21 pi, which was significantly lower (P ≤ 0.01) than G2. On Day 28 pi no oocysts were seen in G5 or G8 faeces. Oocyst output increased in G2 rabbits during the experimental period and reached 148,571.4 opg on Day 28 pi.

Macroscopic examination of G2 rabbit livers showed marked enlargement with large numbers of yellowish nodules of various sizes. There was a significant difference in liver weights between

<table>
<thead>
<tr>
<th></th>
<th>Groups</th>
<th>G2</th>
<th>G5</th>
<th>G8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ± SE</td>
<td>Mean ± SE</td>
<td>%</td>
<td>Mean ± SE</td>
</tr>
<tr>
<td>14 Days After inoculation</td>
<td>30000 ± 4944.1</td>
<td>30002 ± 7600.2</td>
<td>39000 ± 8491.8</td>
<td></td>
</tr>
<tr>
<td>21 Days After inoculation</td>
<td>98571 ± 14285.71</td>
<td>12485.71 ± 18571.43</td>
<td>90.90</td>
<td></td>
</tr>
<tr>
<td>28 Days After inoculation</td>
<td>148571.4 ± 3688.5</td>
<td>0.00 ± 7046.9</td>
<td>90.90</td>
<td></td>
</tr>
</tbody>
</table>

The results of serum biochemical analyses are presented in Table 4 and Figs 4a-g. Compared to the healthy control group (G1), the sera levels of ALT, AST and GGT increased gradually in G2 rabbits throughout the experiment, while the levels dropped significantly in G5 and G8 rabbits by Day 28 pi (P ≤ 0.0). Enzyme levels were similar between these two groups (P ≤ 0.10). The enzyme levels increased in G4 and G7 rabbits, but were not significant when compared to G1 rabbits (P ≤ 0.10).

A gradual and significant increase in TP levels was recorded in G2, G5, and G8 rabbits as compared to G1 rabbits on Day 28 pi. This increase was significantly lower in G8 rabbits than in G2 and G5 rabbits. The TP levels gradually decreased in the uninfected treated groups (G4 and G7) throughout the course of treatment. However, the levels were within the normal ranges with no significant difference (P ≤ 0.10) as compared to the control G1 group.

Direct bilirubin levels in the sera were found significantly increased at Day 21 pi in all of the infected groups (G2, G5, and G8). Bilirubin levels significantly dropped on Day 28 pi in the treated G5 and G8 rabbits and were in the normal ranges in the uninfected treated G4 and G7 rabbits. The levels of alkaline phosphatase peaked on Day 14 pi in all infected groups, and dropped significantly by Day 21 pi. The Hb levels were significantly lower in the infected G2, G5 and G8 rabbits as compared to G1 control rabbits (Table 3, Fig. 3). The liver weight/body weight ratios of the G2 and G5 rabbits were significantly different (P ≤ 0.01). In G8 rabbits, the liver weight/body weight ratios were reduced by Day 28 pi, with a highly significant difference between G8 and G2 rabbits (P ≤ 0.01). The difference between G8 and G5 rabbits was not significant (P ≤ 0.10).
Table 3. Mean (Mean ± SE) of liver weight in all animals groups.

<table>
<thead>
<tr>
<th>Days After Inoculation</th>
<th>Groups</th>
<th>Group G1</th>
<th>Group G2</th>
<th>Group G3</th>
<th>Group G4</th>
<th>Group G5</th>
<th>Group G6</th>
<th>Group G7</th>
<th>Group G8</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L.W.</td>
<td>L.W./B.W.</td>
<td>L.W.</td>
<td>L.W.</td>
<td>L.W.</td>
<td>L.W.</td>
<td>L.W.</td>
<td>L.W.</td>
<td>L.W.</td>
</tr>
<tr>
<td>14 Days After</td>
<td>22.6±</td>
<td>1.52</td>
<td>108±</td>
<td>7.82</td>
<td>25.3±</td>
<td>1.74</td>
<td>22.6±</td>
<td>1.53</td>
<td>106±</td>
</tr>
<tr>
<td>Inoculation</td>
<td>3.71</td>
<td>8.50</td>
<td>2.66</td>
<td>1.76</td>
<td>4.35</td>
<td>9.46</td>
<td>1.91</td>
<td>28±</td>
<td>3.05</td>
</tr>
<tr>
<td>28 Days After</td>
<td>41±</td>
<td>106.3±</td>
<td>10.52</td>
<td>37.3±</td>
<td>2.27</td>
<td>49.3±</td>
<td>3.20</td>
<td>13.86</td>
<td>35.6±</td>
</tr>
<tr>
<td>Inoculation</td>
<td>2.08</td>
<td>6.83</td>
<td>1.33</td>
<td>0.33</td>
<td>13.86</td>
<td>4.54</td>
<td>2.60</td>
<td>2.26</td>
<td>1.76</td>
</tr>
</tbody>
</table>

Relative liver weight = \( \frac{\text{Liver weight}}{\text{Total body weight}} \times 100\%

Figure 4a. Mean serum AST levels.

Figure 4b. Mean serum ALT levels.

Figure 4c. Mean serum GGT levels.

Figure 4d. Mean serum Bil levels.

Figure 4e. Mean serum ALP levels.

Figure 4f. Mean serum TP levels.
Compared to the control G1 rabbits, Hb levels increased significantly more in G8 rabbits as compared to G2 and G5 rabbits (P ≤ 0.05).

**Discussion**

Coccidiosis is one of the most widespread parasitic infections in rabbits causing severe liver damage and high mortality. 1-3. The symptoms characteristic of and associated with hepatic coccidiosis in rabbits include loss of appetite, anorexia, hair loss, diarrhea, yellowish mucous membranes, fatigue, abdomen swelling and significant loss in body weight. In this study, treatment with the recommended dose of myrrh at 500 mg/kg body weight 15, 18, 19, 28-31, 34 resulted in significant recovery of infected and treated rabbits (G5 and G8). All symptoms were eliminated 2 weeks post-treatment, which correlates with previous reports on the efficacy of Mirazid against other parasitic diseases. 14, 22, 36, 47, 49.

Body weight loss is a common symptom of hepatic coccidiosis due to a loss in body fat, which in turn increases fat content in faeces. *E. stiedae* parasites infect the liver and decrease fat absorption and utilization by the infected animal. Liver infections with coccidian parasites damage cells and enzymes that regulate liver physiological functions, and thus interrupt metabolic functions. 67-77.

Treatment of infected rabbits with myrrh (G5 and G8) resulted in a gradually weight gain. This increase in body weight might be due to the lethal effect towards *Eimeria* parasites of the myrrh extracts, thus improving rabbit appetite, food intake, and metabolism. The number of parasite oocysts increased in the G2 rabbit faeces throughout the experiment. This might be due to compromised immunity from *E. stiedae* infection and continuous parasite multiplication.

The crude myrrh suspension and Mirazid were very effective against *E. stiedae* infection, as parasite oocysts were completely cleared from faeces of the infected rabbits by Day 28 pi. This finding suggests that the myrrh extracts reached the parasite in the liver and blocked the progression of its sexual stages, which in turn reduced the number of oocysts in faeces. Though many anti-coccidial drugs have been effective in reducing the number of faecal parasite oocysts detected, some drugs have adverse side effects. These drugs include sulphadimethoxine, narasin (nicarbazin), ivermectin, and aflatoxin. For example, treatment with narasin resulted in slow growth rates, fatigue, muscle pain and hypoglycemia. Other drugs, including demton-S-methyl (DSM), permethrin, methiocarb and diazinone, resulted in changes in histological structures of body organs, such as liver, kidney, and brain, as well as changes in biochemical profiles. Crude myrrh, or extracts such as Mirazid, have been used for the treatment of various health conditions with minor or no side effects. 83-87. Blood biochemical analyses of the treated rabbits (G3, G4, G6, and G7) were within normal ranges following therapy, indicating that the drugs were harmless to the liver.

The increased levels of the serum enzymes AST, ALT and ALP in the infected groups are likely attributable to hepatocellular damage, and the increase in serum GGT to cholestasis. 77, 88-92. Elevated levels of AST and ALT might be an indication of ruptured epithelial lining of the bile duct due to increased numbers of parasite oocysts. 90. The improvement of blood serum enzymes can be attributed to the recovery of hepatic cells and epithelial lining and inhibition of the parasite sexual stages.

Myrrh is a potential source of novel anti-coccidial compounds as evidenced by the complete recovery of the infected rabbits treated with either the crude myrrh suspension or Mirazid (G5 and G8). The recovery included healing of damaged liver tissues, bile ducts, and the main site of infection, and the return to normal levels of many biochemical parameters, including liver enzymes and blood serum proteins following treatment. Treated rabbits displayed increased food intake and metabolism, improved liver function, and likely all metabolic processes dependent on the liver.

The efficacy of myrrh extracts as anti-coccidial treatments is comparable to the efficacy of other medicinal plant extracts such as neem, and other synthetic chemical drugs. The main advantage of myrrh is that it has no apparent side effects, including general body weight and biochemical processes, as reported in this and other studies. 24, 32, 46, 93.

Mirazid, the purified constituent of myrrh, was more effective than the crude suspension. This is likely due to Mirazid containing higher concentrations of the active ingredients commiphoric acid and herrabomyrrhol, which could result in more rapid anti-parasitic effects, therefore significantly reducing the recovery time. This study, together with previous studies, provides strong experimental evidence supporting potential uses of the active ingredients in myrrh, not only for treatment of domestic rabbit coccidiosis, but also against other important microbial and parasitic infections of both veterinary and public health importance.
Table 4. Some biochemical parameters in control and experimental groups.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>7 Days After inoculation</th>
<th>14 Days After inoculation</th>
<th>21 Days After inoculation</th>
<th>28 Days After inoculation</th>
<th>35 Days After inoculation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST  (U/L)</td>
<td>17.73 ± 5.95</td>
<td>27.79 ± 21.0</td>
<td>25.03 ± 5.96</td>
<td>25.0 ± 5.96</td>
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<tr>
<td>ALT  (U/L)</td>
<td>23.56 ± 14.33</td>
<td>20.54 ± 12.56</td>
<td>25.04 ± 12.56</td>
<td>25.04 ± 12.56</td>
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<tr>
<td>ALP  (U/L)</td>
<td>27.79 ± 18.26</td>
<td>25.03 ± 5.96</td>
<td>25.03 ± 5.96</td>
<td>25.03 ± 5.96</td>
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</tr>
<tr>
<td>GGT  (U/L)</td>
<td>25.04 ± 12.56</td>
<td>25.04 ± 12.56</td>
<td>25.04 ± 12.56</td>
<td>25.04 ± 12.56</td>
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<tr>
<td>Bil (UL)</td>
<td>25.04 ± 12.56</td>
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</tr>
</tbody>
</table>

**Hemoglobin** (g/ L) =

<table>
<thead>
<tr>
<th>Parameter</th>
<th>7 Days After inoculation</th>
<th>14 Days After inoculation</th>
<th>21 Days After inoculation</th>
<th>28 Days After inoculation</th>
<th>35 Days After inoculation</th>
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<tbody>
<tr>
<td>7 Days</td>
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<td>32.22 ± 3.43</td>
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<td>35 Days</td>
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<td>32.22 ± 3.43</td>
<td>32.22 ± 3.43</td>
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References


