Products of nitric oxide oxidation and antioxidant vitamins in sheep naturally infested with parasites.

By

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Introduction;

1. Sheep represent an important part of the agricultural economy in Egypt where this species is mostly reared in groups under harsh socioeconomic condition, this leads to low productive and reproductive performance. In addition to helminths parasite causes high mortality and great economic losses, Al-Quaisy et al. Amongst the parasitic disease, end parasites are of greatest importance in sheep where common parasites of sheep and goats include Coccidian, round worms, Tapeworms and liver flukes, Bagly. The humid environmental provides favorable condition for the development and survival of free-living gastrointestinal nematode larvae, Gadahi et al. Gastrointestinal parasite infection is the most important limiting factor of sheep productivity and parasitism has a highly detrimental effect on the sheep industry, so it has always been well recognized because of its high veterinary impact, Mostafa. Young animal do not have great deal of immunity to parasite during the first year and the adult animals are much less susceptible to most parasites, unless they are in poor living condition, Ahmed et al. As The blood sucking parasite, Haemonchus contortus which is found in the abomasums of sheep and goat causes significant blood losses, where each worms removes 0.5 ml / day, so that sheep with 500 Homonchus contorotus may losses about 250 ml / day, Uqauhat et al., resulting in a decrease in erythrocytes, lymphocytes, hemoglobin, body weight and wool growth, Russel et al., Hayat and Hussein, There are important changes in biochemistry of hosts suffering from parasitic infestation depending on the species of the parasites and sites of the host they invade, Russel and McDowell, Ozer et al. Nitric oxide is biological mediator in biochemical reactions and physiologically it is synthesized from the oxidative of
L-arginine to L-citrulline by nitric oxide syntheses employing cofactor NADPH,
Oswald and James. In the host the levels of nitric oxide arise in some pathologic situation where the nitric oxide is oxidized to nitric oxide 2 and nitric oxide 3 within very short time, this short duration in the conversion of nitric oxide to nitric oxide 2 and nitric oxide 3 makes it difficult to accurately measure the concentration of nitric oxide (NO). Therefore by determine the amounts of nitric oxide 2 and nitric oxide 3 the level of nitric oxide can be assessed, Torreilles and Guyerin. Nitric oxide (NO) is produced by a number of different cell types in an inflammatory response to cytokine stimulation by three enzymes called nitric oxide synthesis, inducible (iNOS), endothelial (eNOS) and neural (n NOS). The latter two are active in endothelial and neuron cell while (iNOS) action can be induced in status like inflammation, Frastell et al. While James, reported that nitric oxide play important role in immunologically mediated protection against growing list of protozoan and helminthes parasites, both in vitro and in animal models. In addition to, Rivero recorded that nitric oxide excretes an important selective pressure on parasites. Vitamins are essential to health and must be supplied by food worldwide, vitamins deficiency still result in death either directly or by reducing resistance to disease. Antioxidant vitamins such as E,C and A protected the cells from damage by the free oxygen radicals generated by parasites, Medzyavichyus, also Russel and McDowell recorded that these vitamins have a protective role on the liver.
И . Materials and methods ;

1. Animals ;

1. The study was carried on 50 female non pregnant sheep (weight 30-45 kg, aged 4-5 year old). The sheep were obtained from villages in Assuit city, Egypt, between October 2010 and October 2011, all the sheep field grazed.

2. Forty of the sheep were naturally infected with parasite while the rest number (10 sheep) were clinically and laboratory healthy and used as a control group and these group were treated with ant parasitic agent (albendazole + Rofoxanide) twice at one week long interval and fifteen days following the last treatment the sheep examined by the different parasitological method to indicated that animals were free from any parasites.

2. Samples ;

1. Fecal samples;

Fecal samples were collected directly from all sheep in clean labeled plastic bags and prepared for parasitological analysis.

2. Blood samples ;

1. Two blood samples were collected from jugular vein off all sheep under study as the following.

2. 5 ml blood collected in tube containing EDTA for preparation of blood smear for parasitological examination.

3. 5 ml blood collected in test tube without anticoagulant (EDTA) and left to clot and centrifuged at 2000 g /30 min for serum preparation, the separated serum were a liquated and stored at -20c to be used for biochemical analysis.
3. Parasitological analysis;

1. Fecal samples obtained from sheep under study and analyzed for helminth eggs and larva, cyst and oocysts of protozoa by sedimentation and flotation technique (Vida technique). In addition to that the feces of the infected sheep, egg and oocysts were counted by McMaster technique. Soulsby,\(^{27}\).

2. Blood sample in test tube containing anticoagulant (EDTA) for preparation of blood film were fixed in methyl alcohol and stained with Gimsa stain and examined for microscopic detection of blood parasite and assessment of parasitism.

4. Biochemical analysis;

1. Biochemical analysis carried out on the serum for estimation of nitric oxide concentration according to the modified method of Griess assay, described by Miranada et al.\(^{20}\).

2. Briefly, samples were depronteinized prior to assay. The serum was added to 96% cold ethanol in 1:2 (v/v) and centrifuged for 5 min, incubation for 1/2 h at 4c and the mixture centrifuged for 5 min. The supernatants were used for Griess assay. Analysis was done in a microtitre plate where 100 ul of the supernatant was mixed with vcl3 and fallowed rapidly by addition of the Griess reagent and incubation at 37c for 1/2 hour. The absorbance was measured by a micro plate reader (Multiskan spectrum. Thermo lab systems and Finland) at 540 nm. Nitrite/nitrate concentration was calculated using aNaNo2 standard curve.

3. Antioxidant vitamins (E, C, retinol and its precursor B-carotene) were calorimetrically analyzed using chemical kits (Biodiagnostic, Egypt).

5. Statistical analysis;

The result were analyzed by means ± standard division fallowed by Duncan test using computers software. Person correlation coefficient (SPSS Version12.0 was used
to analyses the correlation between the data where the significance level was set as 
p<0.05.

III. Results ;

1. Clinical examination results;
1. The clinical signs due to parasitic infestation in sheep are ranged from diarrhea, 
emaciation, paleness of the visible mucus membrane, rough the wool and 
submandibular edema,

2. Parasitological analysis results;
1. The laboratory fecal examination of sheep under study revealed that the studied 
sheep were infected with single or multiple infection with parasite as in table (1) 
where eggs of Trichostrongylidae sp, detected by flotation technique, oocysts of 
*Eimeria* and *Dictyoculus filaria* detected by Vidae technique and egg *fasciolia* 
detected by flotation technique while the employing the McMaster technique for egg 
count revealed that the egg numbers of *trichostrongylus sp* were 5600/gm while 
oocysts of *Eimeria* sp were 3900/gm.

2. The parasitological analysis of blood film indicated. There is no blood parasite.

3. Biochemical analysis results .
1. Biochemical parameters of the infested and control sheep are shown in Table,(2,3).

2. The result revealed that the infected group have highly significant increase in 
nitrate and significant increase in nitrite as in table (2) but no significant change in 
retinol level and its precursor B, carotene in any group with significant decrease in 
vitamin E and C as in table (3).
ИИ . Discussion ;

1. The sheep in the present study were under oxidative stress due to the clear change in the oxidant/antioxidant vitamins in the blood.

2. In a number of studies, it has been demonstrated that in the cells of hosts infected with different species of parasites, the amount of reactive oxygen radicals which cause lipid per oxidation are increased. There by causing cell and tissue damage, Stocker et al,30, Smith and Bryant,29, Sarin et al,28.

3. Stimulation of tissue (NO) production is also associated with adverse events such as the production of the potent oxidant peroxynitrite following free-radical reaction with superoxide, Rubbo et al,25.

4. Nitric oxide (NO) is an interesting free radical gas molecule is involved in numerous physiological and path physiological process. The role of nitric oxide (NO) appears controversial because a tissue dysfunction or injury could occur after inhibition of nitric oxide. However high production level (NO) has been suggested as cause of tissue injury, Bahloli et al,4.

5. Antioxidant systems comprised of vitamins have a cellular protective action against oxidative stress which lead to cellular and tissue damage as resulted of parasitic infestation, Mishra et al,17, Dede et al,7 in addition to that. Das et al.5, Saied that the parasite damage the cells which synthesis the molecules carrying the anti oxidative agents, decrease in the number of such cell is natural. Babesia sp cause oxidative degeneration in erythrocytes while Eimeria sp. cause epithelial lesions.

6. Gastrointestinal worm like Trichostongylidea sp cause damage in the cellular lining of the gastrointestinal worm and pulmonary worm like Dictyocaulous Filaria causes damage the cells of lung tissue, Mishra et al,17, Dede et al.,6, Dede et al,7.
In the present study sheep infested with different types of parasites showed clear change in the oxidant /antioxidant vitamins where a highly significant increase in the concentration of (NO) in sheep infected with parasite (p<0.05). This result agreement with Moncada et al., Torreilles and Guyerin, where they reported that the concentration of (NO) synthesized physiologically increase in some pathologic condition and act as a free radicals and this resulted may be attributed to the damage to the cells caused by parasitic invasion. In addition to that, Dede et al., recorded significant increase in the concentration of nitrate in goats infected with parasites. In the present study the level of serum vitamin E and vitamin C level were found to be significantly decrease in the infected sheep(p<0.05) than in the control group and that agreement with Sarin et al., Dede et al., They recorded that the hosts infected with different parasites have a decrease in the concentration of vitamin E and vitamin C and Gameel, reported that serum vitamin C level decrease in sheep infected with Fasciolia sp. In addition to that Dede et al., recorded a decrease in the serum vitamin C in sheep infected with Trichostrongylidae sp.

In the present study no significant change in the concentration level of retinol and its precursor B-carotene between infected and control group and it agreement with Dede et al., but disagreement with Das et al., who recorded a significant decrease in vitamin A in animal infected with parasite. The result of vitamin A may be attributed to that vitamin A has antioxidant activity and play an important role in the body ability to develop an immune response to parasitic infestation where vitamin A, deficient animals have impaired immune response in the parasitic infestation. These findings indicated that the concentration of vitamin E and C is affected by the types of parasites and the hosts they invades.
Y. Conclusion;

1. It was clear that the sheep infested with different types of parasite were under oxidative stress as indicated by significant increase in Nitric oxide (NO) in the blood of these animals.

2. Parasitic infestation have a direct or indirect relationship with the oxidant status of infested sheep, therefore combating of the parasite are essential request for enhancing the productivity of sheep farm.

X. ACKNOWLEDGEMENTS;

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We greatly acknowledge prof. Dr. Ail Hassan Sadiek, Clinical laboratory diagnosis department, College of veterinary medicine, Assuit University, Egypt. For his support.
1. Ahmed M.E, Abdelgadir A.E, Elmalik K.H; Risk factors associated with both blood and internal parasites in dairy farms in Kuku area, Khartoum state. IJAVMS. 2011, 5(4); 405-415


21. Oswald, I. P and James, S. L; Nitric oxide in host defense against parasites, *Methods, 1996; 10; 8-14.


<table>
<thead>
<tr>
<th>Localities</th>
<th>Number of sheep</th>
<th>Parasites</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm, 1</td>
<td>8</td>
<td>2 sheep with <em>Dictycoulus.Filaria</em> +6 sheep with mixed infection (Osterotagia+ strongylus egg)</td>
</tr>
<tr>
<td>Farm, 2</td>
<td>8</td>
<td>3 sheep with Nematodirus+2 sheep with <em>Fasciola.Hepatica</em> +3 sheep with mixed infection(Eimeria+Strogluyus egg)</td>
</tr>
<tr>
<td>Farm, 3</td>
<td>8</td>
<td>1 sheep with Dictycoulus.Filaria+2 sheep with Eimeria+4 sheep with <em>Fasciola hepatica</em></td>
</tr>
<tr>
<td>Farm, 4</td>
<td>8</td>
<td>2 sheep with Fasciola hepatica +6 sheep with mixed infection (Osterotagia +<em>Haemonchus contoratus</em>)</td>
</tr>
<tr>
<td>Farm, 5</td>
<td>8</td>
<td>3 sheep with Fasciola hepatica +2 sheep with Eimeria+3 sheep with mixed infection (Nematodirus +Strongylus )</td>
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</tbody>
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Table; 1 indicated the number of sheep infested with parasit and the type of parasitic infestation.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control sheep (n=10)</th>
<th>Parasitic sheep (n=40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrate(ug/ml)</td>
<td>4.60±2.32</td>
<td>9.01±5.6 **^</td>
</tr>
<tr>
<td>Nitrite(ug/ml)</td>
<td>1.54±1.4</td>
<td>2.62±1.7*</td>
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</table>

*p <0.05

Table; 2, indicated the level of serum oxidant product (NO) in both parasitic and control sheep .

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control sheep</th>
<th>Parasitic sheep</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vitamin C (ug/ml)</td>
<td>0.126±0.037</td>
<td>0.092±0.042*</td>
</tr>
<tr>
<td>Vitamin E (ug/ml)</td>
<td>0.79±0.159</td>
<td>0.61±0.167*</td>
</tr>
<tr>
<td>B-Carotene (ug/ml)</td>
<td>0.43±3.83</td>
<td>0.45±7.12</td>
</tr>
<tr>
<td>Retinol (ug/ml)</td>
<td>0.076±0.013</td>
<td>0.072±0.018</td>
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Table ; 3 , indicated statistical analysis in the level of serum antioxidant vitamins in both parasitic and control sheep.