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Effect of Parasitic Infestation on Oxidant / Antioxidant Status in Buffaloes

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Abstract: Oxidative status is the most important factor that determined health status and productive efficiency of farm animals. Parasitic infestations have major impacts on the productivity and welfare of livestock. The current study was carried out to throw light on the effect of parasitic infestations with different types of parasites; gastrointestinal parasites (*Fasciola gigantica*), blood parasites (*Babesia bovis*) and external parasites (lice) on oxidative status of buffaloes. 40 buffalo cows reared at small holder farms at Lower Egypt were selected for this work. Blood and fecal samples were collected and the body surface was carefully examined for the presence of lice. Fecal samples were microscopically examined and the presence of parasites was recorded. Blood films were examined for the presence of blood parasites. Infestation for Fasciola and Babesia were serologically confirmed by ELISA. Samples were used for determination of the values of some oxidant and antioxidant markers. Results revealed increased values of oxidant and decreased values of antioxidant markers in buffaloes suffering from parasitic infestation as compared with healthy animals especially for values of nitric acid (NO; $P<0.01$). Animals infested with Fasciola showed low value of total antioxidant (TAA; $P< 0.001$), ascorbic acid (ASCA; $P< 0.01$) and catalase (CAT; $P< 0.05$). Babesia infected animals showed low value of ASCA, superoxide dismutase (SOD) and glutathione reduced (GSH-R), ($P< 0.01$) as well as CAT ($P< 0.05$). Lice infested animals had low values of CAT, ASCA and TAA ($P< 0.001$) as well as GSH-R ($P< 0.01$). Lice infestation induced clear changes in the studied markers as compare with other parasites. In conclusion, parasitic infestations, especially with lice have a tight direct or indirect relationship with the oxidant status of infested animals and consequently reflected in poor productivity of farm animals. Therefore combating of these parasites and addition of dietary antioxidants are essential requests for amelioration of productivity of farm animals

Key words: Buffaloes · Fasciolosis · Babesiosis · Lice · Oxidant/antioxidant parameters

INTRODUCTION

Buffaloes represent an important part of the agricultural economy in Egypt and some other developing countries [1]. This species is mostly reared in small holder farms under harsh socioeconomic conditions, this leads to low productive and reproductive performances [2]. The productivity of cattle depends on a complex relationship between breed of animal and availability of food and water in an appropriate environment. A critical component of this productiveness is the issue of management. This includes a variety of factors such as pasture management, nutrition, breeding and animal health. Animal health is one of the factors that affect the efficiency with which animals convert forage into animal protein for human consumption [3].

Parasitic infestation represents an important cause of direct and indirect losses in farm animals [4]. Endoparasites cause significant economic losses and health problems. Fascioliasis caused by liver fluke species of the genus Fasciola, has always been well recognized because of its high veterinary impact [5]. Arthropod ectoparasites are the most ubiquitous life forms affecting animals and commonly affect the daily activity and health status of animals [6]. Although ectoparasites are not critical limiting factors, their presence can affect animal's productivity, as well as economics of production [3]. Animals are infected by a number of parasitic insect and acarine species causing major economic losses in livestock production, intense irritation and skin disease in companion animals, or public health issues, including bites of humans or zoonotic disease transmission [7].

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Insects such as stable flies, house flies, horn flies, face flies, mosquitoes, horse flies, deer flies, cattle grubs and lice as well as ticks and mites are the major external parasites of dairy animals. These pests cause obvious discomfort to livestock and economic effects, especially in heavy populations and usually are easily discerned [8]. Lice may serve as biological or mechanical vectors for various infectious agents. Lice should be evaluated among the broad range of potential vectors of arthropod-borne pathogens [9]. Tick fever is an important disease of cattle whereas Rhipicephalus (Boophilus) microplus acts as a vector for *Babesia bovis, Babesia bigemina* [10]. Tick fever or cattle fever (babesiosis) is economically the most important arthropod-borne disease of cattle worldwide with vast areas of Australia, Africa, South and Central America and the United States continuously under threat. Tick fever was the first disease for which transmission by an arthropod to a mammal [11]. An outbreak of haemoparasitosis in Brown Swiss of various ages was reported. Animals presented fever, severe anemia, jaundice, abortion or premature birth, loss of appetite, decrease milk production and accentuated weight loss in a short period of time [12].

In buffaloes, a tight negative relationship was detected between parasitic infestation and oxidative status of the animals [13]. Stress as a cofactor has been reported to affect the progression and severity of several diseases. Oxidative stress plays an important contributory role in a number of diseases [14]. Oxidative stress is a major problem that results from a number of compounding factors such as pulmonary inflammation and the nutritional inadequacies [15].

The current investigation aimed to through light on the effect of infestation by different types of parasites on oxidant/antioxidant markers in buffaloes under the prevailing Egyptian environmental conditions.

MATERIALS AND METHODS

Animals and Samples Collection: The current study was conducted on buffaloes bred in small holder farms at Lower Egypt (Al-Sharkia governorate) during a period of 2 years (2008-2010). A full case history, owner complains and general health conditions were recorded. The common food available for these animals mainly consisted of Barseem (*Trifolium alexandrinum*), wheat or rice straw and few amount of concentrates mixture. 40 animals were carefully selected for the present study. Animals were chosen to be of the same sex (Buffalo cows), age (3-5 parities), raised under similar managemental conditions, during the same season of the year and apparently free from other diseases. Blood samples were drawn from the jugular vein of each animal into sterile tubes with and without anticoagulants. Blood samples collected on anticoagulant (EDTA) were used for determination of glutathione reduced value [16]. The coagulated blood samples were used for separation of serum (x1500.g; 15 minutes; 4° C) for serodiagnosis of internal and blood parasites. Also, the separated sera were used for determination of some oxidant and antioxidant parameters.

Blood samples from 10 healthy animals which were negative for all types of investigated parasites as well as from 10 animals which showed positive results by fecal examination for *Fasciola gigantic,* 10 animals which showed positive result on examination of blood films for *Babesia bovis* and 10 animals revealing moderate rate of infestation with lice were collected. Positive and negative samples for *Fasciola gigantic* and for *Babesia bovis* were serologically confirmed using ELISA. These samples were used for comparing the effect of infection with gastrointestinal parasites, blood parasites and external parasites on the oxidant/antioxidant status of buffaloes.

Biochemical Analysis: Oxidant/antioxidant markers including malondialdhyde (MDA) [17], nitric oxide (NO) [18], catalase (CAT) [19], superoxide dismutase (SOD) [20], ascorbic acid (ASCA) [21] glutathione-reduced (GSH) [16] and total antioxidant activity (TAA) [22] were colorimetrically analyzed using chemical kits (Biodiagnostic, Egypt).

Statistical Analysis: Data were statistically computed and evaluated according to Sndecor and Cochran [23].

RESULTS

The effect of infestation with different type of parasites on concentration of some oxidant markers in buffaloes is shown in table 1. Generally, values of oxidant markers increased in the blood of infested animals as compared to healthy animals, especially for values of NO ($P<0.01$). Animals infested with lice showed higher values of markers as compared with Fasciola or babesia infected animals.

Table 2 reveals some antioxidant markers in the blood of buffaloes infested with different types of parasites. In general infested animals had lower values for antioxidant parameters as compared to healthy animals. Animals infested with Fasciola showed low value of TAA ($P< 0.001$), ASCA ($P< 0.01$) and CAT ($P< 0.05$). Babesia infected animals showed low value of ASCA, SOD and GSH-R ($P < 0.01$) as well as CAT ($P < 0.05$).

Table 1: Effect of infestation with different types parasites on concentration of some oxidant parameters in buffaloes (Mean± SE)

Oxidant	Healthy Animals	Fasciola infected animals	Babesia infected animals	Lice infected animals
Malondialdhyde (MDA, mmol/L)	1.78 ± 0.09	1.99±0.85	2.01 ± 0.87	2.18±0.34
Nitric oxide (NO, µmol/L)	19.505 ±1.58	21.49±0.29**	20.73±0.67	24.55±1.58 **

**P<0.01

Table 2: Effect of infestation with different parasites on some antioxidant parameters in buffaloes (Mean± SE).

Antioxidant	Health animals	Fasciola infected animals	Babesia infected animals	Lice infected animals
Catalase (CAT, U/ml)	2.38±0.05	0.97±0.36*	0.89 ± 0.04*	0.87±0.03 ***
Ascorbic acid (ASCA, µgm/L)	134.17±5.	94.86±1.37**	98.70 ± 2.35**	98.07±1.74 ***
Superoxide dismutase (SOD,U/ml)	328.16±3.12	318.0±4.04	308.09 ± 2.89 **	316.11±11.12
Glutathione-R (GSH, mmol/L)	6.38±0.11	3.77±.0.12	3.55 ± 0.12**	3.28±0.83 **
Total antioxidant activity (TAA, mmol/L)	1.48±0.04	0.88±0.09***	0.98 ± 0.05	0.67±0.06 ***

*P< 0.05 **P<0.01 ***P<0.001.

Lice infested animals had low values of CAT, ASCA and TAA (P< 0.001) as well as GSH-R (P< 0.01). Lice infestation induced clear decreases in the studied markers as compare with other parasites.

DISCUSSION

Poor reproductive efficiency and parasitic infestation are the most important obstacles for increasing buffalo productivity and cause great economic losses, especially in the developing countries [24]. It can affect the reproductive performance of farm animals through impaired growth rate of young stocks, delayed age at puberty in heifers and prolonged the inter estrous intervals in mature animals [25] as well as negative energy balance and body weight loss [26].

In animals fed on green fodder, natural infection with gastrointestinal nematodes are commonly observed, these internal parasites decreased average daily gain and retard sexual maturation [13]. *Fasciola hepatica*, is a hepatic parasitic infection that affects numerous mammal species, mainly ruminants, in several countries of Europe, Asia and America [25, 28]. The economic significance of fasciolosis is mainly due to direct losses caused by a decrease of weight rate, milking capacity and the confiscation of altered livers in slaughterhouse [25, 27].

Tick fever is an important disease of cattle whereas Rhipicephalus (Boophilus) microplus acts as a vector for *Babesia bovis* and *Babesia bigemina* [10]. Tick fever or cattle fever (babesiosis) is economically the most important arthropod-borne disease of cattle worldwide with vast areas of Australia, Africa, South and Central America and the United States continuously under threat. Tick fever was the first disease for which transmission by an arthropod to a mammal [11]. Babesia is transmitted by ixodid ticks and infection of the host causes a host-mediated pathology and erythrocyte lysis, resulting in anemia, hyperbilirubinuria, hemoglobinuria and possibly organ failure [29].

Over 50 species of ectoparasites infesting cattle throughout the United States [30]. These include 7 species of mites, 22 species of ticks, 6 species of lice and 10 species of flies. Although these ectoparasites are not critical limiting factors, their presence can affect productivity of cattle, as well as economics of production. Losses caused by these parasites result from exsanguination, toxicosis, arthropod-borne diseases and reduced animal production and performance. When assessing the impact of ectoparasites, it is easy to equate mortality, toxicosis and diseases with direct losses. Production traits from cattle under ectoparasite burdens have been variable, ranging from no effect to significant reductions in weight gains [3].

In the present study buffaloes infested with different types of parasites such as Fasciola, babesia and lice showed clear changes in the oxidant/antioxidant markers in the blood. Increased values of oxidative markers have been implicated in tissue damage and the development of chronic diseases [31]. Oxidative stress is an imbalance between radical-generating and radical-scavenging activity, resulting in oxidation products and tissue damage [32]. It results from increased exposure to or production of oxidants, or from decreased dietary intake, synthesis or increased turnover of antioxidants [33, 34]. Stress is known to affect synaptic plasticity, dendritic morphology and induces neurotoxic damage, probably through generation of free radicals [35]. Oxidative stress is a general mechanism whereby free radicals induce oxidative damages and reduce the antioxidant defenses of the biological systems [36]. Overloads of reactive oxygen

species (ROS) including; superoxide, hydrogen peroxide and hydroxyl radical that exceed the capacity of antioxidant systems induce oxidative stress in the body. Accumulating evidence suggested that ROS play a critical role in the pathogenesis of immunological and inflammatory diseases. Excessive exposure to ROS is the hallmark of oxidative stress and leads to damage of lipids, proteins and nucleic acids [37]. Loads of ROS, including superoxide anion and nitric oxide, that overburden antioxidant systems induce oxidative stress in the body. Major cellular targets of ROS are membrane lipids, proteins, nucleic acids and carbohydrates [38]. Exposure to the metal ions can leads to a reduction in cellular antioxidant enzyme activities and lowers cellular defense against oxidative stress [39].

The current increase in MDA values were in agreements with the results of Siemieniuk *et al.* [40]. These authors reported that *F. hepatica* infection enhanced lipid peroxidation, which was reflected by increased level of different lipid peroxidation products such as conjugated dienes (CDs), lipid hydroperoxides (LOOHs), MDA and 4-hydroxynonenal (4-HNE). Liver flukes may cause a decrease in host fertility by altering normal metabolism and/or balance of sex hormones [41]. Kolodziejczyk *et al.* [42] added that, *Fasciola hepatica* fluke-infected cattle rarely demonstrate clinical disease, but subclinical impairment of feed efficiency, growth and fertility can have an important impact on productivity. Rehim *et al.* [43] recorded a significant increase in serum and erythrocyte lipid peroxide levels in *Fasciola hepatica* patients. The influence of stress on the liver is of interest from the clinical point of view because stress plays a potential role in aggravating liver diseases in general and hepatic inflammation in particular, probably through generation of reactive oxygen species [14]. Kolodziejczyk *et al.* [42] reported that, *Fasciola hepatica* infection is accompanied by increased formation of reactive oxygen species. Gameel [44] added that, *Fasciola gigantica* infection in sheep produced liver and lung lesions characterized by damage to blood vessels and parenchymal necrosis. Also, El Samani *et al.* [45] observed that, in the lungs, the lesions were those of parasitic bronchopneumonia. Saleh [36] added that, oxidative stress is a significant feature of chronic *F. hepatica* infection in grazing sheep. On the other hand, Serdar *et al.* [46] observed higher MDA concentration in calves, cows and equines infected with babesiosis and Wright [47] reported changes in lipid composition and metabolism of infected erythrocytes with a consequent increase in membrane lipid peroxidation. No available literatures were traced regarding the effect of lice infestation on oxidant antioxidant status in farm animals, so the condition was explained in other skin disorders. Ozbilge *et al.* [34] and Serarslan *et al.* [48] reported increased LPO and ROS values in cases of cutaneous leishmaniasis, respectively and they attributed the condition to altered enzymatic antioxidant activities. Vural *et al.* [49] concluded that such affection more likely induces the endogenous antioxidant system. Camkerten *et al.* [50] reported that, lipid hydroperoxide level, total oxidant status and oxidative stress index in dogs with sarcoptic mange were higher than the control. They suggested a possible relationship between oxidant/antioxidant imbalance and sarcoptic mange infestation in dogs.

It was clear that, infected animals in the current study were under oxidative stress as indicated by increases in NO in the blood of animals in relation to fasciolosis, babesiosis and lice infestation. Similar results were previously recorded by Xiao *et al.* [51] who recorded that, NO in sera of goats artificially infected with *Fasciola hepatica* was significantly changed post-infection. Also, Commins *et al.* [52] and Mostafa [53] reported that, erythrocytes infected with Babesia merozoites induce nitric oxide synthase (iNOS) transcription and stimulate the production of NO by the activated macrophages in cattle [54]. The increased production of these free radicals leads to augmented oxidative stress as evidenced by high levels of erythrocytic lipid peroxidation products [55]. In bovine babesiosis caused by *B. bovis*, the infection involves production of interleukin-1β, interleukin-12, gamma interferon (IFN-γ), tumour necrosis factor-α (TNF-α). These mediators activate mononuclear phagocytes/macrophages to release reactive nitrogen intermediates [56, 57]. The mechanism of cytotoxicity was dependent on and directly proportional to, the production of NO [58]. Shoda *et al.* [56] stated that, the tick-transmitted hemoparasite *Babesia bovis* causes an acute infection that result in persistence and immunity against challenge infection in cattle that control the initial parasitemia. Resolution of acute infection with this protozoal pathogen is believed to be dependent on products of activated macrophages (Mphi), including inflammatory cytokines and NO and its derivatives. *B. bovis* stimulates inducible nitric oxide synthase (iNOS) and production of NO in bovine Mphi and chemical donors of NO inhibit the growth of *B. bovis* in *vitro*. Goff *et al.* [59] added that, innate immunity in cattle exposed to hemoparasites is spleen-dependent and age-related. On the other hand, Chaudhuri *et al.* [60] observed oxidative damage in dogs naturally infected with

B. gibsoni, low level of blood iron, zinc and copper seems to have an additional role in the genesis of anaemia and oxidative stress. Superoxide anions are increased in erythrocytes parasitized with *B. gibsoni* and oxidative damage, due to lipid peroxidation, might be caused in host erythrocytes by the parasite [61]. On the other hand, Dimri *et al.* [62] concluded that, the skin parasite Psoroptic mange infestation increases oxidative stress and decreases antioxidant status in sheep. Saleh *et al.* [63] added that, dromedary sarcoptosis is accompanied by a state of oxidative stress process, which increased by increasing the area of infestation and may contribute to the pathogenesis of the disease.

A number of antioxidants and trace minerals have important roles in immune function and may affect health in transition dairy cows. Susceptibility to infectious disease is dependent on the integrity of the immune system [64]. The antioxidant markers were glutathione peroxidase activity and concentrations of zinc, selenium, vitamin A, vitamin C, vitamin E [65, 66] and SOD [66]. The glutathione (GSH)-oxidant defence system protects the erythrocytes and leucocytes from oxidative damage [67]. Vitamin E and beta-carotene are important cellular antioxidants. Selenium (Se) is involved in the antioxidant system via its role in the enzyme glutathione peroxidase [68]. Superoxide dismutase (SOD) is one of the most important antioxidant enzymes present in all oxygen-metabolizing cells. This enzyme eliminates toxins from the body [69]. Catalase is reported to be one of the target antigens for autoantibodies in various pathologies [70].

Results of the current study revealed, decrease in concentration of some antioxidant markers; CAT, ASCA, SOD, GSH-R and TAA in the blood of buffaloes infested with fasciolosis, babesiosis and lice infestation as compared to healthy animals. These results agreed with the results of Rehim *et al.* [43] whereas they recorded a significant decrease in glutathione levels as well as in glutathione peroxidase (GPX) and SOD activities in *Fasciola hepatica* patients as compared to their corresponding control values. Değer *et al.* [71] observed that, diminution of antioxidant abilities resulted in enhanced oxidative modifications of lipids and proteins. In cattle infected with *Dictyocaulus viviparus* the activity of GPx in the group with distomatosis were higher than in the control group. They added that, the Cu, Zn-SOD, CAT activities and the GSH, vitamin C concentrations in the cattle infected with *Dictyocaulus viviparus* were significantly lower than in the control group. On the other hand, Siemieniuk, *et al.* [40] observed a decrease in antioxidant capacity of the Wistar rat livers, manifested by a decrease in total antioxidant status (TAS).

The present decreases in the antioxidant markers; GSH and ASC concentrations was in consistent with the results of Bicek *et al.* [72] who reported that hosts infected with Babesia species had low levels of GSH.

Lice may serve as biological or mechanical vectors for various infectious agents [9]. Lice should be evaluated among the broad range of potential vectors of arthropod-borne pathogens [73]. It was recorded that animals infested with ectoparasites show disturbance of oxidant/antioxidant balance [74-77]. Neutrophiles and macrophages produce reactive oxidants such as hydrogen peroxide, hypochlorite and oxygen radicals which have potent cytotoxic effects on parasites as well as other pathogenic organisms [78]. On the other hand, Dimri *et al.* [62] recorded decrease in antioxidant enzyme activities and trace mineral concentrations in buffaloes infected with sarcoptic mange. They also suggested that sarcoptic mange in buffaloes is associated with compromise in antioxidant defense and oxidative stress may play important role in pathogenesis. Also, it was found that free radicals induce or contribute to adverse effects on the skin through erythema, edema, wrinkling, inflammation, autoimmune reactions, hypersensitivity and keratinization abnormalities [79]. Moreover, Lipid peroxidation can be harmful for skin due to alternations in the membrane structure and permeability [80]. On the other hand, Burns *et al.* [73] reported that, erythrokinetic and plasma protein turnover, were carried out on young Ayrshire calves naturally infested with the long-nosed sucking louse *Linognathus vituli*. Throughout the trial none of the animals lost their appetite. Body weight gain was greater in the uninfected animals but not significantly. The infestation did not cause the animals to become anemic, but plasma iron turnover rates were decreased and there was a significant increase in the red cell survival time. The circulating volumes of red cells and plasma were not significantly affected and the fractional catabolic rate of albumin was not markedly altered. Kocyigit *et al.* [81] reported that, serum essential trace elements Se, Zn, Cu and Fe concentrations and their related enzymes Cu-Zn SOD, GSH-Px and CAT activities change in cutaneous leishmaniasis cases. The changes may be a part of defense strategies of organism and are induced by the hormone like substances.

In conclusion, parasitic infestations, especially with lice have a tight direct or indirect relationship with the oxidant status of infested animals and consequently reflected in poor productivity of farm animals. Therefore combating of these parasites and addition of dietary antioxidants are essential requests for amelioration of productivity of farm animals

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