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Impact of Adding Antioxidant Vitamins (A or E) with antihelmintic Treatment on Naturally Gastrointestinal Nematode Infected Ewes

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SUMMARY

The present study was aimed to examine the oxidative stress/antioxidant status in naturally infected ewes with gastrointestinal nematodes(GIN) after use of antioxidant vitamins such as vitamin A or E with antihelmintic treatment (ivermectin) .A total of 12 Barki ewes were chosen naturally infected with GIN ,aged between (1.5 – 2.5) years, weighted between (35 -38 kg). All ewes were treated with 2ml/50kg BW ivermectin. They were divided into three groups (n=4). The first group was non-vitamin treated (the control group), the second group, was orally treated with vitamin A ($0.5X10^5$ IU/ewe/day)for four weeks, the third group was orally treated with vitamin E (75mg/ewe/day)also for four weeks. Fecal samples were collected from rectum of each ewe before the beginning of the trial and weekly after treatments. Degree of infestation was performed by fecal egg counting (FEC) and identified by fecal culture technique. Blood samples were collected from each ewe for biochemical analysis of serum lipid peroxide–Malondialdehyd (MDA), Nitricoxide (NO), total-antioxidant capacity (TAC) and glutathione–S-transferase (GST). Ewes were weighed every two weeks till the end of the study. Four weeks post-treatment, MDA significantly ($p < 0.05$) decreased in their levels in both vitamin treated groups in comparison to the control one, meanwhile NO showed no alterations in their levels in both treated groups in comparison to the control one all over the expermental period. Four weeks post treatment, there was significant ($p < 0.05$) increase in the mean value of TAC in vitamin E treated group comparing with the other groups. While there were significant increase in GST level after the second and the fourth weeks in vitamin E treated group. It was concluded that vitamin E should be used at least 2 weeks with other antihelmintic protocols in order to obtain a more effective and earlier cure against GI parasites in

infected sheep for improving their health & reproductive performance.

INTRODUCTION

Gastrointestinal nematodes (GIN) represent a major economic burden to agriculture communities worldwide, causing infections resulting in anaemia, weight loss and ultimately death in small ruminants (Van Rossum *et al.*, 2004). It has also remained health problem for man and domesticated animals in the tropical and subtropical regions of the world (Fakae and Brophy, 2001). Since Gastrointestinal nematodes are internal parasites causing inflammation which lead to stress on the host, the detection of oxidative stress/antioxidant status are important biomarkers for host parasite interactions, although there are several biochemical studies on the sheep parasitism few reports are available regarding the antioxidant status during natural infection of gastrointestinal nematodes (Guzel *et al.* 2008).

Oxidative stress may result from an imbalance between reactive oxygen species (ROS) and anti-oxidant level (Lightboy *et al.*, 2001). It is well known that ROS are produced by several pathological conditions and cause cellular damage such as lipid peroxidation and protein oxidation. Lipid peroxidation is a well- established mechanism of cellular injury and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxide derived from polyunsaturated fatty acids is unstable and can decompose to form a complex series of compounds, these include reactive carbonyl compound, which is the most abundant malondialdehyde (MDA). Therefore, measurement of MDA is widely used as an indicator of lipid peroxidation.

Increased level of free radicals products has been associated with a variety of chronic diseases in both human and animals Rojo-Vazquez *et al.*, (1981) Romero *et al.*, (1998). There are other important change in the biochemistry of hosts suffering from parasitic invasions depending on the species of the parasite and the sites of the hosts they invade (Aksakal and Ozer, 1987). Nitric oxide (NO) is a biologic mediator in biochemical reactions, and physiologically, it is synthesized from L-arginine by NO synthase employing co-factor Necotinamide Adenine Dinucleotide Phosphate Oxidase (NADPH). In the host the levels of NO arise in some pathologic situations (Torreilles and Guyerin, 1995).The biological oxidative effect of free radicals on lipids and proteins are controlled by a spectrum of antioxidant (Serdar *et al.*, 2007).

The antioxidant status of tissues can be described by the analysis of single component in the defense system against ROS, as well as by the determination of total antioxidant capacity (TAC) (Kankofer *et al.*, 2005). Glutathione S-transferases (GST) are multifunctional enzymes which play a key

role in cellular detoxification. The enzymes protect cells against toxicants by conjugating them to glutathione, thereby neutralizing their electrophilic sites and rendering the products more water soluble. The glutathione conjugates are metabolized further to mercapturic acid and then excreted. These classes are comprised of both cytosolic and microsomal enzymes (Brophy *et al.*, 1994)

Antioxidant vitamins such as E and A protect the cells from damage against free oxygen radicals generated as a result of parasitosis (Medzyavichyus *et al.*, 1989) and also they have a protective role on the liver (Russel and McDowell, 1989). Some studies reported that parasitic infestation predispose animal to vitamin and meniral deficiency (Deger *et al.*, 1997 and Tanritanir *et al.*, 2009). With chemicals no longer effectively control of nematodes in several parts of the world, understanding the host-parasite relationship at the mucosal feeding surface is important in identifying new therapeutic approach. There are presently no commercial vaccines available for gastrointestinal nematodes infections and the most effective method of control is a combination of pasture management and the use of anthelmintic (Van Rossum *et al.*, 2004).

So, our target in this trial was to test the hypothesis of increased activity of defense system protecting tissues by vitamins against the damaged effect of free radicals in ewes infected with GIN. Our approach was to investigate the impact of use a combination of commercial anthelmintic drug plus vitamin A or vitamin E on infected ewes weight and find out the elevations in their antioxidant status before and after treatment by these combinations as a new therapeutic approach.

MATERIALS AND METHODS

This trial was investigated in Animal Reproduction Research Institute (ARRI)-sheep farm from April to May 2010, 12 Barki ewes were chosen naturally infected with GIN aged between (1.5 – 2.5) years, weighted between (35 -38 kg). Ewes were housed in an open yard system under natural light and temperature and were fed with balanced ration according to ARRI management, water *ad libitum*. All ewes were treated by 2ml/50kg BW of ivermectin (Bomectin^{R}, product of BoMAC Laboratories, 1% W/V). Ewes were divided into three groups (n=4), the first group (control group) left without vitamin treatments, the second group, was orally treated with vitamin A ($0.5X10^{5}$ IU/ewe/day) (Kahira Pharma. & ind. co. Cairo, Egypt) for four weeks according to Hidiroglou and Batra (1996), the third group was orally treated with vitamin E (75mg/ewe/day) (produced by Pharco Pharmaceutical Alex) for four weeks according to Hidiroglou *et al.*, (1990).

Parasitological examination:

Faecal samples were collected from rectum of each ewe before the beginning of the trial and weekly after treatments. Samples were examined for the presence of GIN eggs by saturated concentration floatation technique using saturated sodium chloride solution (Soulsby, 1982). Degree of infestation was performed by fecal egg counting (FEC) using McMaster technique according to Urquhart *et al.* (1988). A coproculture (faecal culture for identification of the infected larvae) at 26°C for (7-10) days was performed and the larval identification determined according to Georgi *et al.* (1985).

Clinical examination:

All ewes included in this study were subjected to clinical examination; the infected ewes showed depression of appetite, pale mucous membranes, anemia and impairment in the wool quality, inspection of ewes revealed that no history or signs of systemic diseases.

Sampling: 10 ml of blood were collected from the jugular vein of each ewe into plain vaccutainers for serum separation and stored at -20°C for biochemical analysis of serum lipid peroxide (Malondialdehyde) MDA (Satohk, 1978), Nitric oxide (NO) (Montgomery and Dymock, 1961), total antioxidant capacity (TAC) (Koracevic and koracevic, 2001), glutathione–S-transeferase (GST) (Habig *et al.*, 1974), all the previous parameters were estimated by colorimetric methods. Ewes were weighed every two weeks till the end of the study.

Statistical analysis:

Values were expressed as mean ± SE. Statistical comparisons between the means of different experimental groups during the experimental trial were made with completely randomized one way ANOVA "Student_ Newman_ Keuls test" by COSTAT program version one. A probability "P" value of <0.05 was assumed for statistical significance according to Snedecor and Cochran (1980).

RESULTS

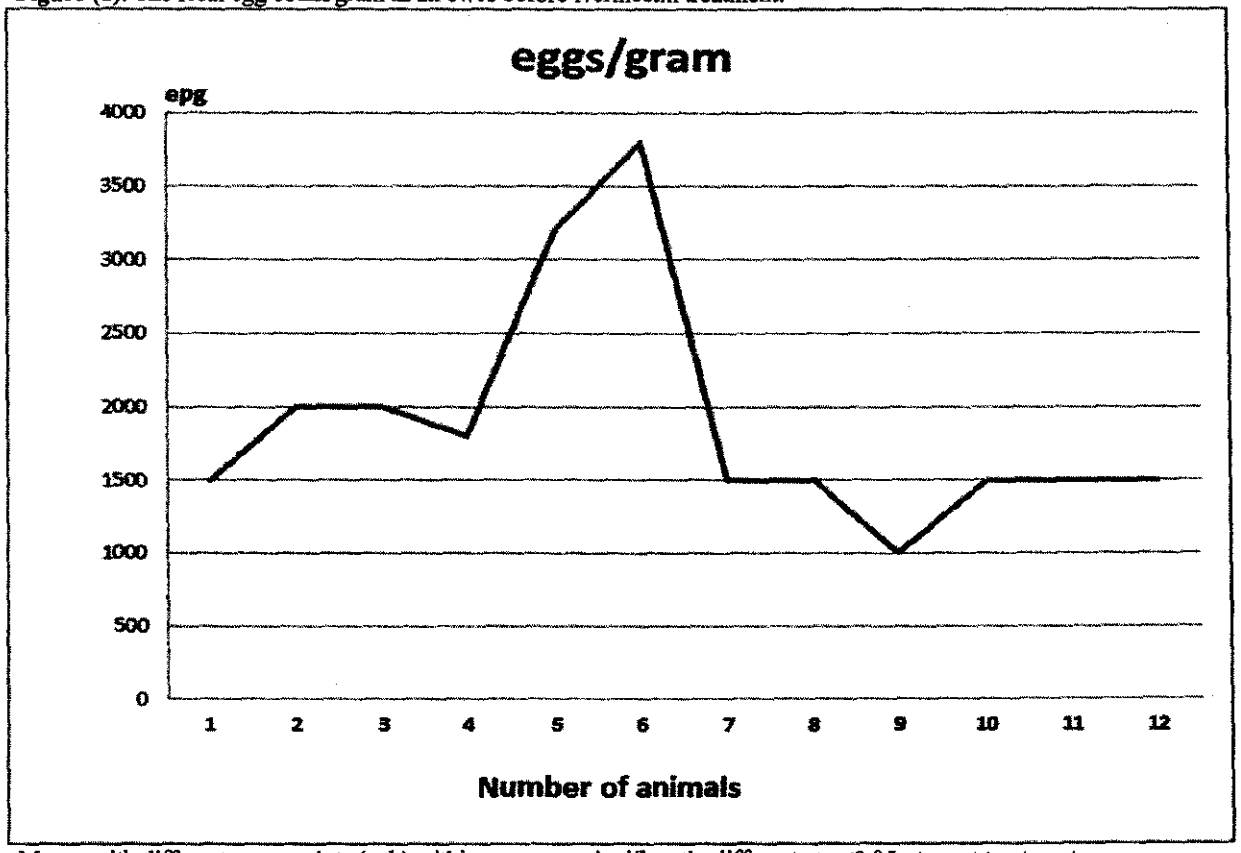
Examination of faecal samples before the treatment revealed that all ewes were naturally infected with different species of gastrointestinal nematodes (GIN), FEC ranged from (1000-3800) with average 1900 epg fig (1). The commonest gastrointestinal parasites were *Haemonchus sp., Trichostrongylus sp., Chapertia sp., Trichuris sp.*

Values of free radical serum levels (MDA and NO) (table 1) showed non-significant changes before and two weeks post treatment. Four weeks post-treatment, MDA showed significant decrease ($p<0.05$) in their levels in both treated groups in comparison to the control one, as they recorded (8.37 ± 0.15, (6.06 ± 0.16) and (5.59 ±0.28) for control, vitamin A and vitamin E treated groups respectively. Meanwhile NO

showed non-significant decrease in their levels in both treated groups in comparison to the control one in all experimental period. The results of Total antioxidant capacity and Glutathione-S-transferase levels are illustrated in tables (2); TAC showed non- significant changes in ewes' serum level before and two weeks post treatment for both vitamins treated groups. Two weeks post treatment, GST levels showed significant increase ($p<0.05$) in vitamin E treated group in comparison to the other groups, the levels recorded (0.1 ± 0.002), (0.1 ± 0.003) and (0.11 ± 0.01) for control, vitamin A and vitamin E treated groups respectively. Four weeks post treatment, there were significant increase ($p<0.05$) in the mean values of TAC in vitamin E treated group versus the control one; the TAC value recorded (0.22 ± 0.02), (0.31 ± 0.02) and (0.29 ± 0.01) mmol/L for control, vitamin A and E groups respectively, also GST levels showed significant increase in the the fourth week in vitamine E group only, GST values were (0.08 ± 0.004), (0.01± 0.004), (0.93 ± 0.002) mmol/L for control, vitamin A and E respectively.

Concerning the body weights of ewes under investigation (table 3), showed non-significant changes ($p<0.05$) in the body weight of all groups along the experimental period

Figure (1): The fecal egg count/gram in all ewes before ivermectin treatment:



Means with different superscripts (a, b) within a row are significantly different at $p<0.05$ pt: post treatment

Table(1): Effect of antioxidant vitamin A/E treatment on MDA &NO levels in GIN infected ewe:

Item	MDA µmol/ml			NO µl/L		
	control	Vit A group	vit E group	control	Vit A group	vit E group
day zero	7.68 ± 0.5[a]	8.88 ± 0.4[a]	7.93 ± 0.2[a]	31.03 ± 1.04[a]	31.21 ± 1.8[a]	28.98 ± 0.9[a]
2 weeks pt	7.4 ±0.2[a]	6.96 ± 0.4[a]	6.78 ± 0.5[a]	43.69 ± 2.3[a]	34.17 ± 4.9[a]	45.92 ±1.2[a]
4 weeks pt	8.37 ± 0.2[a]	6.06 ± 0.2[b]	5.59 ±0.3[b]	41.82 ± 1.6[a]	37.29 ± 2.01[a]	40.05 ±1. 3[a]

Table (2): Effect of antioxidant vitamin A/E treatment on Total antioxidant capacity and glutathione-S- transferase levels of GIN infected ewes:

Item	TAC mmol/L			GST mmol/L		
	control	Vit A group	vit E group	control	Vit A group	vit E group
day zero	0.2 ± 0.01[a]	0.2 ± 0.01[a]	0.21 ± 0.01[a]	0.1 ± 0.003[a]	0.1 ± 0.003[a]	0.1 ± 0.01[a]
2 weeks pt	0.24 ±0.02[a]	0.29 ± 0.02[a]	0.26 ± 0.01[a]	0.1 ± 0.002[a]	0.1 ± 0.003[ab]	0.11 ± 0.01[b]
4 weeks pt	0.22 ± 0.02[a]	0.31 ± 0.02[a]	0.29 ± 0.01[b]	0.08 ± 0.004[a]	0.1± 0.004[a]	0.93 ± 0.002[b]

Means with different superscripts (a, b) within a row are significantly different at $p<0.05$ pt: post treatment

Table (3): Effect of antioxidant vitamin A/E treatment on body weight of GIN infected ewes:

Item	body weights(kg)		
	control	vit A group	vit E group
before treatment	39 ± 2.45^a	40.37 ± 1.55^a	39 ± 2.45^a
4 weeks post treatment	38.25 ± 1.89^a	38.5 ± 2.76^a	41 ± 0.71^a

Means with different superscripts (a, b) within a row are significantly different at $p<0.005$

DISCUSSION

GIN infections are chronic perversive infection that contributes worldwide to morbidity and mortality in humans and livestock (Koski and Scott, 2001). In general GIN infection reduces nutrient availability to the host through both reductions in voluntary feed intake and/or reduction in the efficiency of absorbed nutrients although the mechanisms of the depression in appetite haven't been fully elucidating (Dynes *et al.*, 1998). In the current study, four weeks post treatment, there were decreases in the levels of MDA (lipid peroxide) in ewes treated with antioxidant vitamins A and E in comparison to the control group; the high metabolic stress (due to parasitic infestation) lead to increase the free radicals values in the blood of ewes, the excessive lipid peroxidation in plasma and cells arise from many factors or diseases lead to excessive formation of NADPH (Necotinamide Adenine Dinucleotide Phosphate Oxidase), which in turn promote lipid peroxidation in the presence of cytochrome P-450 system (Ahmed *et al.*, 2010). (Serdar *et al.*, 2007) demonstrated that in the cells of host infected with different species of parasites, the amount of reactive oxygen radicals -which cause lipid peroxidation- are increased causing cell and tissue damage.

Our results strongly suggested that one of the main reasons for this decrement could be due to vitamin treatment which increases activity of defense system protecting tissue from free radical damage as mentioned by Sarin *et al.*, (1993). In addition, Ruder *et al.*, (2008) reported that ROS (reactive oxygen species) in living cells are formed continuously as a consequence of biochemical reactions; within the mitochondrial respiratory chain and external factors, OS (oxidative stress) induces lipid peroxidation, occur when the generation of ROS and other radical species exceed scavenging by antioxidant as a result of excursive production of ROS and/or inadequate intakes or increased utilization antioxidants, Antioxidant vitamins are compound capable of disposing,

scavenging or suppressing the formation of ROS.

The current study revealed that, four weeks post treatment, there were increase in TAC levels in vitamin E treated group in comparison to the other groups. This result agreed with that of Lightboy *et al.*, (2001) on parasitized sheepand goat, and explained that the oxidative stress may result from an imbalance between ROS and antioxidant levels, in addition Kankofer *et al.*, (2005) mentioned that the antioxidant status of tissues can be described by the analysis of single component in the defense system against ROS as well as by the determination of TAC. On contrary, TAC measurement does not represent the sum of activities of antioxidants; it could be used for clinical diagnosis as it is an easy and less time consuming procedure.

This study demonstrated that glutathione-S-transferase (GST) level significantly increased in vitamin E supplemented group than in the other groups. GST is associated with the establishment of parasitic nematode infection within the gastrointestinal environment of the mammalian host as well as their levels have been shown to increase in parasitic helminthes during chronic parasitic infection. Some researchers have attempted to correlate this over-expression with the ability of GST to detoxify immune-initiated cytotoxic products of lipid peroxidation (Brophy *et al.*, 1994). Meanwhile, Van Rossum *et al.*, 2004) analyzed a new GST from the sheep infected with strongylid and *H. contortus* showed that this GST does not appear to have abroad immune defense or drug metabolism role but possibly has a more focused detoxification function within the nematodes.

Das et al., (1994) and Ginsburg and Atamina, (1994) have proposed that, since parasites cause damage in the cells which synthesize the molecules carrying the antioxidant agents, a decrease in the number of such cells is natural, as well as GI worms cause damage in the cellular lining of GI tissues. In addition Dede *et al.*, (2002) found significant lower vitamin E in the parasitized goats than in the non-infected ones. Also Sarin *et al.*, (1993) found that in hosts infected with different parasites, the concentrations of vitamin E fell in comparison to healthy controls. So in the present investigation, antioxidant systems comprising vitamins A and E specially vitamin E have a cellular protective action against oxidative stress resulting in cells, organs and tissue damage caused by parasitic invasion which is in agreement with Dede *et al.*, (2000).

Kozat *et al.*, 2007 examined the influence of parasitism and vitamin A and E supplementation on infected ewes, and mentioned that GI parasites may impair intestinal absorption of vitamins and attributed it to lower feed intake especially in

proteins which might explain the non-significant changes in the ewes body weight in our study. As well as GIN infection reduces hydrolysis in the abomasum when pH is elevated by the infection may contribute to reduce some trace elements availability and absorption which associated with anorexia and weight loss (Albers *et al.*, 1990) and (Suleyman *et al.*, 2007).

Conclusion:

In the final course of our investigation we came to the convention that vitamin E should be used at least for two weeks to obtain a more effective and earlier cure against GI parasites in sheep infected with it together with other treatment protocols that will improve ewes production and reproduction.

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تأثير إضافة الفيتامينات المضاده للأكسدة(أ&هـ) أثناء العلاج بمضاد الطفيليات فى النعاج المصابه طبيعياً بالديدان الإسطوانية

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*قسم بيولوجيا التكاثر معهد بحوث التناسليات الحيوانيه بالهرم-الجيزه/ مصر

أجرى هذا البحث بغرض متابعة تأثير العلاج بالفيتامينات أ أو هـ إلى جانب العلاج بمضاد الطفيليات على مستوى بعض الاجسام الشاردة و مضادات الأكسدة فى النعاج المصابة طبيعياً بالديدان الإسطوانية . أجريت التجربة فى مزرعة معهد بحوث التناسليات الحيوانية بالهرم.

تم إستخدام عدد 12 نعجة برقى بالغة متوسط أعمارها من 1.5 الى 2.5 سنة ومتوسط أوزانها من 35 الى 38 كيلوجرام. وتم إختيارها مصابة طبيعيا بالديدان الأسطوانية بعد التشخيص الطفيلى. تم حقن جميع النعاج تحت الجلد بالايفرميكتين 2 ميلليجرام لكل 50 كيلوجرام من وزن كل نعجة. قسمت النعاج الى ثلاثة مجاميع كل مجموعة تحتوى على ٤ نعاج، المجموعة الاولى وهى الضابطة المعالجة بالإيفرميكتين فقط، المجموعة الثانية عولجت بفيتامين أ ($0.5X10^5$ وحده دوليه لكل نعجه يومياً) و المجموعة الثالثة بفيتامين هـ (75 ملليجرام لكل نعجه يومياً) لمده أربع أسابيع لكلا المجموعتين الثانيه و الثالثه.

أخذت عينات البراز من كل نعجة على حدة لفحصها طفيليًا أسبوعيًا قبل وبعد العلاج، وحددت درجة الاصابة بعد البيض مع تحديد انواع الديدان المصابه بها النعاج. تم تجميع عينات الدم من جميع النعاج كل على حدة وذلك لقياس مستويات بعض الأجسام الشاردة (المالون داي ألدهايد و النيتريك أوكسيد) و مضادات الأكسده (مضادات الأكسدة الكلية والجلوتاثيون اس ترانسيفيراز) وتم وزن جميع النعاج كل اسبوعين حتى نهاية التجربه. بعد أربعة أسابيع من العلاج كان هناك نقص معنوى فى مستوى المالون داي ألدهايد في كل من المجموعتين الثانيه والثالثه، فى حين انه لم يكن هناك تغير معنوى فى مستوى النيترك أوكسيد طوال التجربة فى ذات المجموعتين مقارنة بالمجموعة الضابطة. مستوي الجلوتاثيون إس ترانسفيراز زاد معنوياً فى المجموعة الثالثه بعد الأسبوع الثانى والرابع من التجربة فى حين زاد مستوى مضادات الأكسدة الكلية معنوياً فى المجموعةالثالثه بعد الأسبوع الرابع من التجربه.

لذلك توصى هذه الدراسة بإستخدام فيتامين هـ لمده أسبوعين على الأقل إلى جانب الطرق العلاجية المعتادة للديدان الإسطوانية لتحسين درجة تأثيرها إلى جانب الإسراع من شفاء النعاج المصابه لتحسين حالتها الصحيه و كفائتها التناسليه.