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Effect of Zinc and Selenium Fortification of Diet on Hematological and Biochemical Parameters of Fasciola Infected Ewes

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AN experiment was designed to investigate the effect of fortifying rations with Zinc and/or Selenium on physiological performance of fasciola infected ewes. Biweekly blood samples, for 8 weeks period, from thirty naturally fasciola infected Barki ewes were taken and analysed for packed cell volume (PCV), haemoglolbin concentration (Hb), erythrocyte and total leucocyte and it's differential counts. Three Wintrobe indexes (MCV, MCH and MCHC) were also calculated. Blood plasma Zinc and Selenium were determined. Feacal parasite egg was also counted. Results indicated that adding Selenium alone or in combination with Zinc to the ration significantly increased erythrocyte counts but decreased leucocyte counts as compared to adding Zinc alone. The decrease in total leucocytes by adding Selenium or Selenium with Zinc was reflected in a significantly decrease in lymphocytes, neutrophils, eosinophils and basophilic cells. Monocytes was significantly decreased by Zinc fortification as compared to other treatments. PCV as well as Hb were significantly increased by Selenium and Zinc/Selenium fortification. Results also showed that MCV and MCH decreased but MCHC increased significantly by treatments not Zinc fortification alone. Ewes body weight and plasma Zinc were not significantly different as a result of different treatments. Blood plasma Selenium was increased after Selenium fortification. Throughout the 8 weeks of the experimental period, erythrocytes, PCV, Hb, MCHC, plasma Zinc, Selenium and body weight had steadily increased. Total and differential leucocytic counts except that of the basophils as well as MCH and MCV were decreased.

KEY WORDS : (Zinc, selenium, heamatology, Fasciola infested sheep)

Fascioliasis is a widespread disease of cattle and sheep (Daws, 1963 and Soulsby, 1973). The prevalence of infection with *Fasciola sp.* among cattle had also been reported by many researchers (Combanas, 1966 and Babenkass *et al.*, 1968). The infection reduces milk yield and meat quality as well as decreases body weight gain, wool quality and feed conversion efficiency (Pantilouris, 1965; Duell, 1975 and Hope *et al.*, 1977). The parasite was also found to cause decrease in erythrocyte counts, haemoglobin level, albumin and inorganic phosphorus (Jennings *et al.*, 1965; Morshkin *et al.*, 1965; Holms, 1968 and El-Magdoub, 1970). On the other hand leucocytosis associated with eosinophillia as well as high blood plasma protein were reported by (Balbo *et al.*, 1973 and Issoref *et al.*, 1977). High plane of nutrition, however may counteract the damage caused by the flukes. Infected animals fed on high protein rations with or without trace elements level and packed cell volume (Dimtrov *et al.*, 1978 and Oacly *et al.*, 1979). Therefore the objective of the present study was to investigate the effects of fortifying rations with zinc and/or selenium on the haematological picture of fasciola infected ewes.

Material and Methods

Animals, feeding and management

Thirty naturally fasciola infected Barki ewes of the same age and weight were used in the present study. Ewes were left to graze on berseem *Trifolium alexandrinum)* and offered a daily allowance of about 450 gm. of straw and 150 gm. of cotton seed cake per day, (Morrison, 1957) in addition to 1% ordinary salt and 2% calcium carbonate of rations weight. Animals were divided randomly into 3 groups of 10 each which were kept in separate barnes. Ewes in the first group were singly drenched daily with 60 ppm Zinc as zinc oxide. Ewes in the second group were drenched daily with 0.15 ppm selenium as sodium selenite per ewe while those in the third group were drenched with both zinc and selenium at the same previously mentioned levels per ewe.

Data, sampling collection and analysis

Biweekly blood samples, for eight weeks period, were taken from each

animal by Jagular puncture into heparinized test tubes. Blood samples were analyzed for packed cell volume (PCV), haemoglobin concentration (Hb) and erythrocyte and total leucocyte counts, within 30 min. of sampling. PCV and Hb were determined according to the method described by Oser (1965) and erythrocytic and leucocytic counts were conducted on an Ao Bright-line haemocytometer. Differential leucocytes distribution was determined using Leishman's stain blood film.

Three wintrobe indexes

Mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated using the formulas proposed by Schalm (1965). Samples were then centrifuged at 886 Xg for 20 min. to obtain plasma samples. Blood trace minerals in plasma samples determined using the methods of Mille *et al.* (1967) and AOAC procedure (1975). Feacal parasite egg counts were carried out as described by Lapage (1965). Ewes were weighted every two weeks to the nearest 100 gm.

Data was analyzed as 3 × 4 factorial arrangement of treatment in completely randomized design as described by Snedecor and Cochran (1969). Specific differences between treatments were tested using Duncan's Multiple Range Test.

Results and Discussion

Results indicated that adding selenium alone or in combination with zinc to the ration significantly ($P < 0.01$) increased the number of erythrocytes in blood of infected ewes as compared to adding zinc alone (Table 1). Roland *et al.* (1979), Said and Handols (1980) and El-Sayed *et al.*, (Under Publication) found that infection with fascioliasis decreased erythrocyte counts. The last authors working on the same bread of infected ewes, found that RBC reached a low value compared with all of our values (Table 1). These results might give an indication about the beneficial effects of given trace minerals to infected ewes. It was also found, throughout the present experiment, that as period of treatment progressed their was a significantly ($P < 0.01$) progressive increase in blood erythrocyte counts. However, the rate of increase differed between treatments which was reflected in a significant ($P < 0.01$) treatment by period interaction. As it can be seen in Fig. (1), ration supplemented with zinc alone did not improve the number

TABLE 1. Overall mean values of haematological parameters, trace minerals, body weight and intensity of infection in sheep throughout the experimental period.

	Treatment ± S.E.		
	Zinc	Selenium	Zinc + Selenium
Haematological parameters:			
Erythrocyte counts ($\times 10^6/mm^3$)**	6.980 ± 0.32	8.030 ± 0.44	8.170 ± 0.16
Leucocyte counts ($\times 10^3/mm^3$)**	9.970 ± 0.74	9.240 ± 0.90	9.170 ± 1.01
Lymphocyte counts ($\times 10^3/mm^3$)**	6.130 ± 0.03	5.820 ± 0.03	5.830 ± 0.03
Monocyte counts ($\times 10^3/mm^3$)**	0.113 ± 0.03	0.126 ± 0.06	0.131 ± 0.06
Neutrophil counts ($\times 10^3/mm^3$)**	3.020 ± 0.20	3.020 ± 0.30	2.820 ± 0.35
Eosinophil counts ($\times 10^3/mm^3$)**	0.415 ± 0.08	0.380 ± 0.03	0.351 ± 0.04
Basophil counts ($\times 10^3/mm^3$)**	0.480 ± 0.05	0.416 ± 0.03	0.396 ± 0.03
Packed cell volume (PCV)**	26.410 ± 0.48	26.960 ± 0.40	27.520 ± 0.55
Haemoglobin (gm/100 ml)**	8.690 ± 0.02	9.850 ± 0.04	9.840 ± 0.07
Wintrobe indexes:			
MCV (u^3)**	37.880 ± 0.15	33.870 ± 0.21	34.170 ± 0.30
MCH (uug)**	12.490 ± 0.04	12.470 ± 0.06	12.010 ± 0.08
MCHC (%)*	33.290 ± 0.01	36.480 ± 0.08	35.320 ± 0.09
Trace minerals:			
Zinc (ppm)	0.290 ± 0.04	0.303 ± 0.07	0.306 ± 0.07
Selenium (ppm)**	0.022 ± 0.04	0.024 ± 0.01	0.024 ± 0.004
Body weight (kg):	28.060 ± 0.04	28.330 ± 0.06	28.910 ± 0.06
	41.160 ± 3.00	41.160 ± 3.58	30.280 ± 3.34

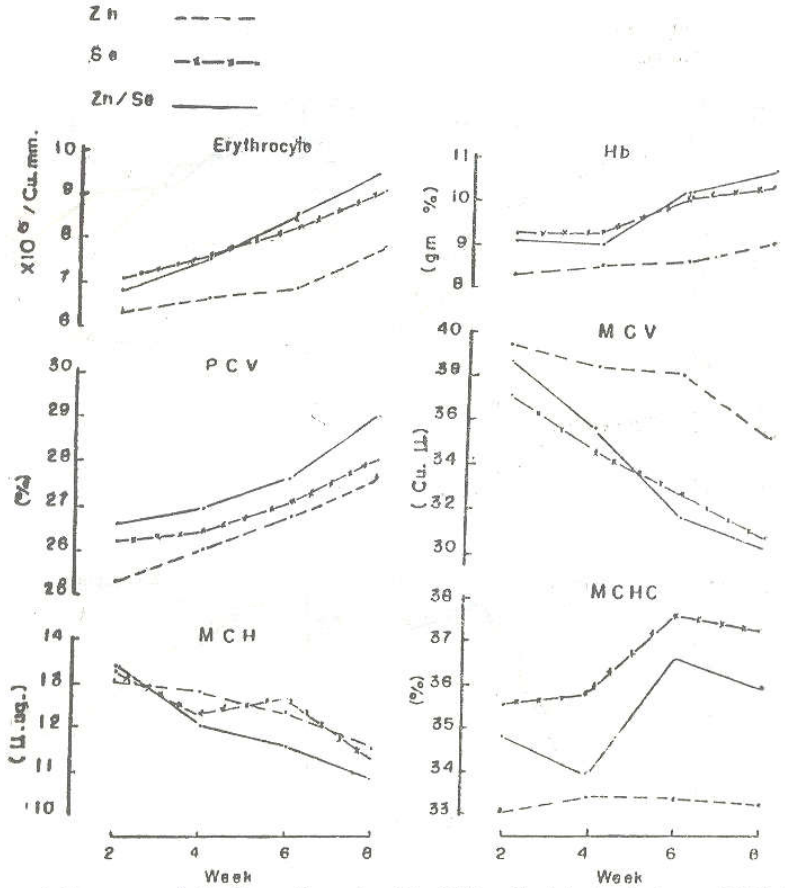

Figure 1. Responses of blood erythrocytes, Hb, PCV and wintrob indexes of infected ewes of ration fortified with Zinc (.....), Selenium (—X—X—) and both (——) throughout eight weeks of experimentation.

of erythrocyte throughout the experiment as much as selenium or zinc with selenium supplementations.

Results also indicated that adding both zinc and selenium to the ration significantly ($P < 0.01$) decreases the number of leucocytes than did zinc

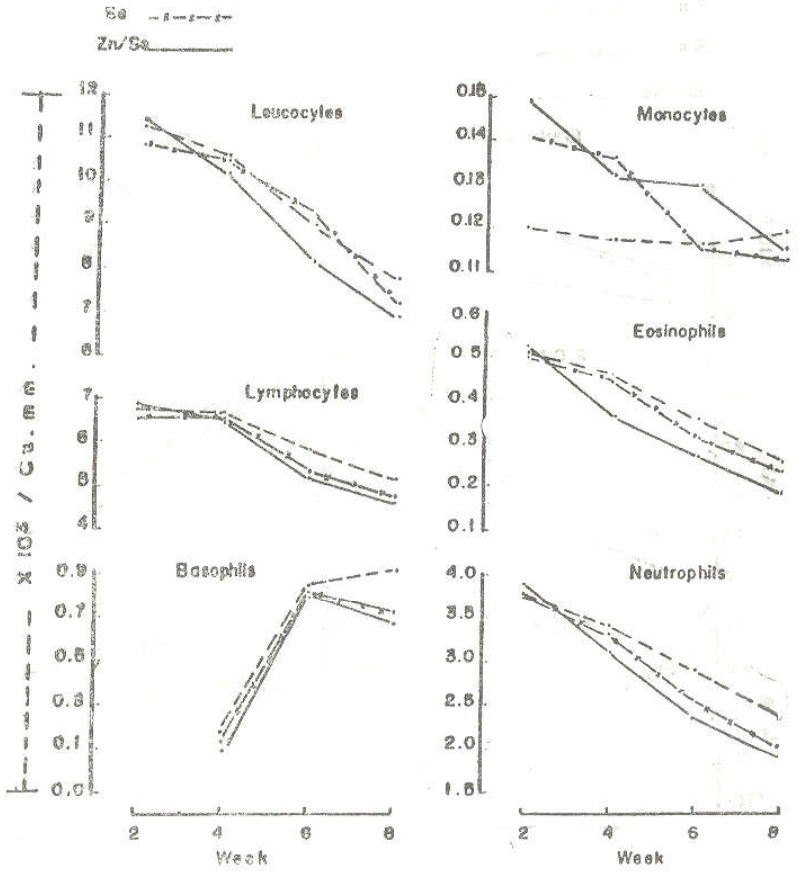

Figure 2. Responses of blood total leucocyte and it's differential counts of infected ewes of ration fortified with Zinc (— — —), Selenium (—X—X—) and both (——) throughout eight weeks of experimentation.

or selenium alone (Table 1). Selenium, however, was more effective than zinc in decreasing the blood leucocyte counts of infected ewes. The number of leucocytes also decreased significantly as experimental period progressed in all of the three groups of ewes (Fig. 2). However, the decrease was only drastic after the fourth week of experimentation. It was also found that

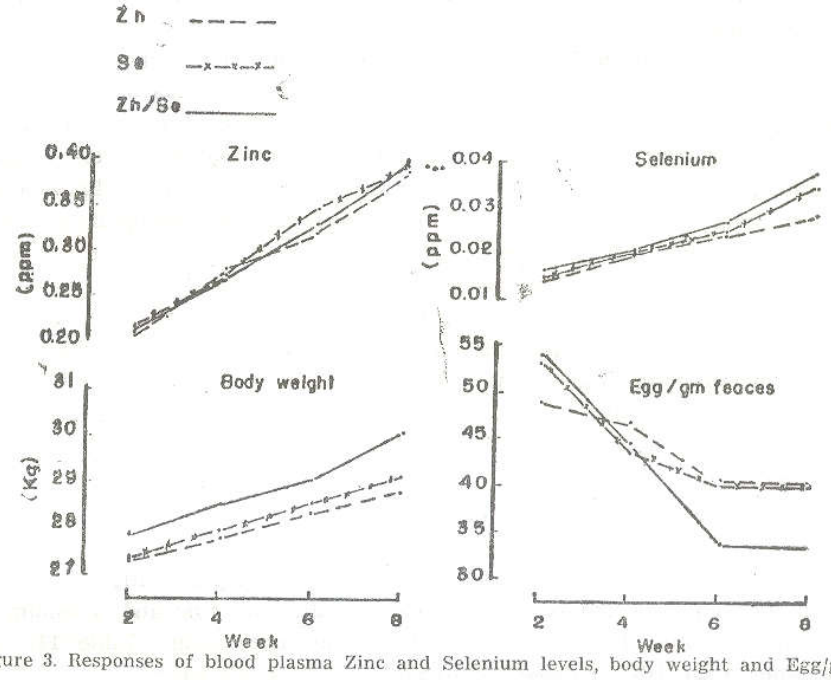

Figure 3. Responses of blood plasma Zinc and Selenium levels, body weight and Egg/gm feaces of infected ewes of ration 'fortified with Zinc (— — —), Selenium (X—X—X—) and both (———) throughout eight weeks of experimentation.

adding both zinc and selenium to the ration was more effective over the duration of the experimentally period than adding any of these trace minerals alone. Lymphocyte counts of infected ewes fed ration fortified with selenium alone or selenium with zinc were significantly ($P < 0.01$) lower than that of the infected ewes fed ration with zinc alone (Table 1). It was also found that the number of blood lymphocytes and monocytes of all groups of ewes, fed different ration decreased significantly ($P < 0.01$) and gradually over the successive experimental periods (Fig. 2). The decrease in lymphocyte count was more pronounced when ration fortified with both trace minerals (Fig. 2) reflecting a statistically significant ($P < 0.01$) interaction between treatments and duration of the experimental period. The present authors found in another study (Under publication) that ewes lymphocytes increased following fasciola infection and explain that by increasing the inflammatory tissue of the ewes. Thus, reduces the lymphocyte numbers by feeding dif-

ferent trace minerals in the present study might be due to the reduction in the inflammatory reaction. Results of the present study showed that ration fortified with both minerals zinc and selenium significantly ($P < 0.01$) decreased the number of all of the granulocytic cell counts of the infected ewes (Table 1). differences between values of infected ewes fed ration fortified with zinc or selenium were not significant but they were higher significantly ($P < 0.01$) than those for ewes fed ration with both minerals. It was found that neutrophilic cell counts and that of eosinophils of all of the three groups of infected ewes fed on different fortified rations decreased all over the experimental period. However the decrease was more pronounced with those animals fed on ration fortified with both trace minerals (Fig. 2). It was also found that during the first two weeks of the experimental period basophilic cell was not found in the blood of all groups of ewes. However, during the fourth throughout the eighth week of the experimentation basophilic cells started to appear and increased gradually although values decreased little between the sixth throughout the eighth week of experimentation (Fig. 2).

Present results also revealed that PCV increased significantly ($P < 0.01$) when infected ewes fed on ration fortified with both zinc and selenium as compared to those fed rations with zinc or selenium alone (Table 1). On the other hand, Hb increased significantly ($P < 0.01$) when ewes fed selenium alone or with zinc. Difference between the last two groups was not significant (Table 1). Values of PCV and Hb were significantly ($P < 0.01$) and gradually increased throughout the eighth week of experimentation in all of the three groups of the infected ewes, although, it was more pronounced when ewes fed ration with both trace elements (Fig. 1). The increase in PCV values and Hb concentrations with erythrocyte counts by adding trace minerals especially zinc and/or selenium suggested that these minerals might enhance the impaired erythropiotic activity in the infected ewes. Results indicated that feeding both trace minerals decreased significantly ($P < 0.01$) MCV, MCH of infected ewes as compared to other groups. However, feeding ration with selenium alone significantly ($P < 0.01$) decreased MCH as compared to those fed ration with zinc alone (Table 1).

On the other hand, MCHC was significantly ($P < 0.01$) higher in the blood of infected ewes fed selenium fortified ration as compared to other treatment groups (Table 1). It was also found that as experimental period progressed up to the eighth week of experimentation there were a signi-

ficantly ($P < 0.01$) and gradually decreased in MCV and MCH but increased in MCHC (Fig. 1). Values of MCHC of infected ewes fed ration with zinc were more or less constant at a loC value throughout the duration of the experimental period (Fig. 1). MCHC for the other two groups, however, showed a little decrease at the end of the experimental period (Fig. 1). Differences between blood plasma zinc of infected ewes fed rations fortified with different trace minerals were not statictically significant (Table 1). On the other hand, ewes fed on ration fortified with selenium alone or along with zinc had a significantly ($P < 0.01$) higher plasma selenium as compared to ewes fed only zinc (Table 1). The levels of both of those minerals in the infected ewes were low during the first two weeks and then increased gradually by feeding the different trace minerals. Symons *et al.*, (1984) suggested that the deleterious effects of fasciola infections may be due to the impairement of protein metabolism within the animal body which leads to zinc difficiency based on reduced plasma zinc level.

Body weight differences between the three groups of ewes due to feeding zinc and/or selenium in rations were not statistically significant (Table 1). However, body weight of all groups were increased progressively ($P < 0.01$) as experimental period progressed but the substantial increase was observed with those offered both trace minerals (Fig. 3). Present results, however agreed with those of Horton (1977) and Symons (1984). Results also indicated that infected ewes fed ration with both selenium and zinc had significantly ($P < 0.01$) low eggs/gm feaces than did those had only zinc or selenium in their ration (Table 1). Differences between the later two groups was not significant. The intensity of the infection in the three experimental groups of infected ewes decreased progressively (Fig. 3). The decrease of the intensity of the infection was more pronounced when fed ration with both trace minerals.

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تأثير اضافة كل من الزنك والسيلينيم على بعض صفات الدم الهيماتولوجية والبيوكيميائية للنعاج البرقى المصابة بالديدان الكبدية

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صممت هذه التجربة لدراسة تأثير اضافة كل من الزنك أو السيلنيم أو الاثنين معا الى عليقة النعاج البرقى المصابة بالديدان الكبدية . وأخذت بعض الصفات الهيماتولوجية والبيوكيميائية لدم هـذه النعاج وكذلك وزن الجسم وعدد بيض هـذه الديدان فى الروث كمقياس لدراسة تأثير هـذه المعادن . أخذت عينات من الدم طوال فترة التجربة أسبوعيا ولمدة ثمانية أسابيع من ثلاثون نعجة برقى مصابة بالديدان الكبدية وقدر فى هـذه العينات قيمة الهيماتوكريت ومستوى الهيموجلوبين وعدد كرات الدم الحمراء والبيضاء وأنواعها المختلفة . وقدر كذلك حجم كل كرة دموية حمراء وما تحتويه من الهيموجلوبين وما يشغله الهيموجلوبين من مساحة كـل كرة حمراء . كما فصلت البلازما وقدر فيها نسبة الزنك والسلينيم وحسب أيضا الزيادة فى وزن الجسم وكذلك عدد البيض فى الروث .

ودلت النتائج على أن اضافة السلينيم بمفرده أو اضافته مع الزنك الى العليقة قد أدى الى الزيادة معنويا فى عدد كرات الدم الحمراء ونقص فى عدد كرات الدم البيضاء وذلك اذا ما قورن باضافة الزنك فقط . وهذا النقص فى عدد كرات الدم البيضاء انعكس على الأنواع اللمفاوية والمتعادلة والحامضية والقاعدية أما بالنسبة للنوع الأحادى فقد حدث نتيجة اضافة الزنك فقط بالمقارنة باضافة الزنك مع السيلينيم وقد أظهرت النتائج حدوث زيادة معنوية فى قيمة الهيماتوكريت ومستوى الهيموجلوبين نتيجة اضافة السيلينيم مع الزنك أيضا أو اضافة السيلينيم بمفرده . وكذلك حجم الكرة الدموية الحمراء وما تحتويه من الهيموجلوبين ولكن لم تحدث هذه الزيادة باضافة الزنك بمفرده .

أما بالنسبة لوزن الجسم وما تحتويه البلازما من الزنك فلم تكن هناك اختلافات معنوية على مستوى المعاملات . ولكن حدث زيادة فى مستوى السيلينيم بالبلازما وذلك باضافة السيلينيم الى العليقة . وعموما خلال الثمانية أسابيع وهى فترة التجربة قد حدث زيادة معنوية فى كل من كرات الدم الحمراء وقيمة الهيماتوكريت ومستوى الهيموجلوبين ونسبة ما تحتويه كل كرة من الهيموجلوبين ووزن الجسم ومستوى الزنك والسيلنيم فى البلازما . وحدث نقص معنوى فى عدد كرات الدم البيضاء وكل أنواعها ما عدا النوع القاعدى فالنقص كان غير معنوى وكذلك حجم الكرة الدموية الحمراء وما تحتويه كل كرة من الهيموجلوبين فكان مقدار النقص معنوى .