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Relationship Between Plasma Minerals and Nematode Infection Load in Moghani Ewes

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Abstract: The objective of this study was to evaluate some of plasma minerals concentrations during naturally acquire gastrointestinal nematode infection (GIN) in Moghani ewes. A total of 35 Moghani ewes aged 7-12 months and 33±2 kg body weight were randomly selected which their infection had confirmed by McMaster flotation method. The animals were divided into 3 groups according to egg count per gram of faeces (EPGs). Blood samples were taken from the jugular vain of animals after 7 hours fasting period and concurrently faecal samples were taken from the rectum of each animal. The animals of the experiment had not received any anthelmintic drugs at least 4 months prior to sampling. The plasma was used for determination of Ca: P ratio and Calcium (Ca), Phosphorous (P), Iron (Fe) and Magnesium (Mg) concentrations by spectrophotometric method using the commercial kits. According to our results, there was no significant difference between groups concerning all the studied parameters. Also, we observed a significant positive correlation between the EPGs and Ca: P ratio ($P<0.05$, $r=0.350$). Our results indicate that the load of GIN infection (EPGs < 850) had no significant effect on plasma Ca, P, Fe and Mg concentrations in Moghani sheep.

Key words: Nematode infection · Egg per gram of faeces · Plasma minerals · Moghani ewes

INTRODUCTION

Gastrointestinal parasites (GIP) are one of the major problems in animal breeding management. Apart from the other impacting factors on breeding, the preventing of GIP is necessary for improvement of animal performance particularly in ruminant. Uncontrolled parasitic infections cause economic losses in livestock industry, directly [1]. The degree of economic losses is dependent on some agents such as load of infection, age, gender, animal nutrition and physiological state. The GIN infection has a major effect on the good revenue from grazing sheep. A common feature of many GIP infections is diarrhea, scours, rough hair coat, bottle jaw, pale mucous membranes (eyelids and gums), anemia and death [2]. Various parasitic agents have different effects and clinical signs in sheep. For example, *Haemonchus placei* cause anemia, *Ostertagia ostentagi* reduces feed intake and also, *Cooperia ancophora* and *Cooperia punctata* affect protein metabolism a nd reduce efficiency of feed in cattle [2- 4].

Endoparasitism especially nematodes are the main parasites in small ruminants, mainly in sheep. These pathogenic helminthes live in digestive tract particularly in abomasum and intestine of the host animal [5], gastrointestinal parasites cause incomplete proliferation of epithelial cells, disorder in mineral absorption and modification in ion channels in different segments of the small intestine [5]. These changes can lead to reduce in absorption and retention of sub-nutrient and minerals such as Ca, P, Fe and Mg. Simultaneously, high load of GIN infection cause decrease of mineral absorption of the diet [3].

The structural failures of gastrointestinal tract due to some of the GIN infection cause blood loss which followed by Fe deficiency in chronic status. Mineral substances play a key role in metabolism, growing periods and functions of reproduction system in animals [6, 7]. Iron is indispensable for the normal activity of the nervous, vascular and immunological system [8]. This element is a component of many proteins such as haemoproteins, catalase, peroxidase, respiratory chain cytochromes, cytochrome P-450 and may be act as an

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activator of redox enzymes and involved in regulation of immune responses, aerobic and anaerobic energy metabolism [8, 9]. Its deficiency induces a reduction of Fe tissue reserves at first, followed by a drop in its plasma concentration. Anemia often occurs after heavy deficiency [10]. Although, Fe deficiencies, expect in young ruminants and milk fed calves, do not occur in ruminants [11].

Calcium is one of the most important minerals which exist in all of the body tissues. In plasma, it exists in three forms: bound to protein (mainly to albumin), ionized and bound with other onions (e.g. citrates, Po_4 and lactate) [12]. Calcium is required for many normal body functions e.g. muscle contraction, appetite, hormones secretion, glycogen metabolism and might be acts as a second messenger. Its deficiency can lead to severe osteomalacia and osteoporosis in parasitized sheep. Major factors that determine serum Ca concentrations are include age, serum proteins and absorption from the intestine and resorption or deposition in bone [12, 13].

Phosphorus is other important element which is necessary for generation of the ATP, NADPH and phosphoproteins. Similar to the Ca, it is involved in regulation of immune responses. Additionally, it has a key role in all metabolic reactions [9, 14]. The physiological ratio of Ca: P is 2:1 to 1:2 and its change is harmful for animals [9]. Calcium and P can be absorbed in all the parts of small intestine [13, 14]. The GIN infection can reduce absorption of Ca and P by altering in epithelial cells [15]. Intestinal nematode infection e.g. (*Trichostrongylus Spp.*), cause marked villous atrophy in the anterior of small intestine [16]. Moreover, physiological processes such as renal clearance, absorption in intestine, resorption in bone, growth and hormonal regulation affect plasma P concentration [12, 13, 17].

Magnesium is a cofactor for large number of enzymes (e.g. kinase) and has catalyzing effect on many biological reactions such as oxidative phosphorylation [9, 14, 18]. It is required for ruminal microbial function appetite, immune responses, inflammation and also energy for cell function [13, 18]. Similar to Ca, plasma Mg is distributed into three fractions: free Mg, bound to proteins and non- protein anions [12]. Major factors that determine the plasma Mg are including: absorption in gastrointestinal tract, excretion in kidney, milk and hormonal regulation [12, 19- 21]. Additionally, overall variations in level of above mentioned parameters are affected by some other endogenous or exogenous factors such as species, breed, age, gender, season, the regional soil characteristics, nutritional and physiological different phases of reproductive cycle status (e.g. lactation and pregnancy) in ruminant [9, 19-26]. There is no information about the variation and correlation between the plasma minerals concentrations and the load of nematode infection in Moghani ewes.

The main propose of this study was to determining correlation of the plasma Ca, P, Fe and Mg levels with different load of GIN infection in Moghani ewes.

MATERIALS AND METHODS

Experimental Location and Animals: This experiment was carried out at the Moghan plateau, Ardabil province, northwest of Iran (30°24'35.47" N and 48°18'12.36" E and 98M altitude) in summer 2010.

At first, we found flocks of grazer Moghani sheep which had not received any anthelmintic drugs at least 4 months prior the experiment and their infection confirmed by McMaster flotation method. Among them, a total of 35 non-pregnant, 7-12 months old and 32±3 kg body weight of grazer Moghani ewes were randomly selected which their infection had confirmed previously. The animals were divided into 3 groups according to EPGs. During the study, all the animals fed *ad libitum* by grazing at Moghan plateau and had free access to water.

Sampling Procedur: All animals were giving 7 hours fasting period before faecal and blood sampling. Faecal samples were taken from the rectum of each animal and kept in individual sampling containers under 4°C. Blood samples (10 ml) were collected from the jugular vein using disposable syringes containing heparin as an anticoagulant. Blood samples were centrifuged at 750× g for 10 minute and plasma was separated and stored at -20°C until used.

Laboratory Analyses: Faecal egg count was determined using McMaster flotation technique [27] at laboratory of parasitology at the Islamic Azad University, Shabestar branch. Shabestar, Iran. The majority sizes of the obvious gastrointestinal eggs in the examined faecal samples using scaled microscope were between 70 - 90 μm. These eggs size were consistent with ones of *Trichostrongylus, Ostertagia, Haemonchus* and *Nematodirus* genus.

Plasma Ca, P, Fe and Mg concentrations were determined by colorimetric method using auto analyzer (Mindray- BS-200, Germany) at clinical pathology laboratory at the Islamic Azad University, Shabestar branch. Shabestar, Iran.

Table 1: The values of faecal egg count and plasma minerals in naturally nematode infected Moghani ewes (Mean ± SD)

Group	N	EPGs	Fe (µg/dl)	Mg (mg/dl)	Ca (mg/dl)	P (mg/dl)	Ca:P
1	12	319±22	171.21±52.66	2.26±0.24	9.45±0.88	6.56±1.12	1.47±0.26
2	13	404±43	162.62±53.86	2.35±0.26	9.46±0.70	6.93±1.47	1.43±0.35
3	10	752±97	175.04±40.30	2.27±0.26	9.68±0.48	6.32±1.94	1.70±0.44

SD=Standard Deviation

EPGs= Egg per gram of faeces

Table 2: Correlation coefficients and statistical analysis between EPG and plasma minerals in naturally nematode infected Moghani ewes

		EPG	Ca	P	Fe	Mg	Ca:P
EPG	Pearson Correlation	1	0.112	-0.143	0.156	-0.193	0.350*
	Sig. (2-tailed)		0.522	0.411	0.370	0.268	0.039
Ca	Pearson Correlation		1	-0.174	-0.252	0.158	0.418*
	Sig. (2-tailed)			0.317	0.144	0.363	0.012
P	Pearson Correlation			1	0.046	-0.043	-0.903**
	Sig. (2-tailed)				0.794	0.808	0.000
Fe	Pearson Correlation				1	-0.124	-0.110
	Sig. (2-tailed)					0.480	0.529
Mg	Pearson Correlation					1	0.025
	Sig. (2-tailed)						0.886
Ca:P	Pearson Correlation						1
	Sig. (2-tailed)						

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

Statistical Analysis: The data were analyzed by one - way analysis of variance and Pearson's correlation method using SPSS / ver. 17 software. Duncan's multiple range test was used to detect significant differences between the means. All values are shown as mean ± standard deviation (SD).

RESULTS

The values of the studied parameters in different groups are presented in Table 1. Correlation coefficients and statistical analysis between all the parameters in Moghani ewes are shown in Table 2. According to the results there was no significant difference between groups concerning the studied minerals. Additionally, there was not a significant correlation between EPGs and all the studied parameters, except to Ca: P ratio *($p<0.001$, $r=0.350$)*. Although, there was a negative but not significant correlation between EPGs with the plasma P (*$r = -0.143$*) and Mg (*$r = -0.193$*) concentration *($p>0.05$)*.

DISCUSSION

In the current study, the level of plasma Ca was higher than the values reported previously in non-infected lambs [23] and lower than the other values reported in non-infected Moghani ewes [9], in GIN infected sheep [28] and goat [29]. This result indicated decreasing effect of GIP on the plasma Ca concentration compared to non-infected sheep. But, no significant difference was observed among groups concerning plasma Ca level. The negative effect of GIN on plasma Ca level compare to non-infected animals was consistent with previous reports in sheep [28, 30] and was inconsistent with the other report in infected goat [29]. The level of P was higher than the values reported in GIP infected sheep [28], goat [29] and other breeds of Iranian ewes [20]. We found no reducing effect of GIP on plasma P level compare to non-infected sheep which was consistent with the previous report [29] which had observed in goats and was disagree with previous report in sheep [28]. In the present study, the level of Mg was lower than the values reported in other breeds of Iranian sheep [20] and was agree with the values reported in non-infected Moghani ewes [9] and in GIP infected sheep [28]. Additionally, the level of Fe was consistent with the reported value in infected sheep [28] and was higher than the previous reports in Moghani ewes [9] and goats [29].

Current results indicated that the GIN had no decreasing effect on plasma Mg and Fe concentration compared to the values reported previously in non-infected Moghani ewes [9]. In the present study, it was observed a non-significant negative correlation only between EPGs and the Mg and P values. The GIN infection impairs the mineral metabolism and absorption

(especially Ca, P and Mg) from the ruminant intestine in ruminant and also body tends to maintain the Ca: P ratio about 2:1 to 1: 2 ratios [21, 30]. Therefore, the changes of Ca and P levels seem normal. Furthermore, none of the plasma studied parameters were affected by the load of nematode infection and lake of difference in the studied minerals between groups suggests that the nematode parasitism (EPGs ranged: 250-850) apparently was not severe enough to impair the nutritional status of the nematode infected animals. As aforementioned, overall endogenous factors (e.g. species, breed, age, gender, hormonal and physiological states) and exogenous factors such as nutritional states and composition, season, the region soil characteristics and also the rate and stage of development, number of hatched eggs and related variations involved in inconsistent of our results with the other previously reposts in sheep and other ruminants [9, 12, 17, 20, 31-34]. As these factors have complex effect on the plasma minerals concentrations in sheep, so the exact explanations of these finding is not possible at this time. Although, our results were cleared that the GIN infection had non-significant effect on all the studied parameters in Moghani ewes (with the EPGs < 850); and other factors especially age, gender, nutrition and physiological status may be affect on results.

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