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ORIGINAL ARTICLE

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Effect of experimental nematode infection on serum mineral concentrations in growing lambs

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Abstract The objective of the present study was to determine the possible effect of gastrointestinal nematodes upon serum mineral concentrations of lambs. Twelve male lambs were used. Lambs were randomly assigned to 2 groups (n = 6): Group 1 infected with gastrointestinal nematodes and Group 2 as controls. Lambs of Group 1 were infected with a single dose of 15,000 L_3 larvae of GI nematodes (*Haemonchus*, *Teladorsagia*, *Trichostrongylus*, *Cooperia* and *Oesophagostomum–Bunostomum*). Blood samples were collected from the investigated animals individually every 2 weeks. However, the differences in serum macro-minerals (Ca, Mg, P, K, and Na) among groups were not significant. Although the differences in serum macro-minerals among groups were not significant and the iron serum concentration remained unaltered, the gastrointestinal parasitism reduced significantly/substantially the serum copper levels.

Keywords Gastrointestinal nematodes · Macro-minerals · Copper · Iron · Lambs

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Introduction

Many of the macronutrient minerals and trace or micronutrient mineral elements have been found to be "essential" for the higher forms of animal and human life and also very important for the health and production parameters of farm animals. They exert their role in the organism in various ways, as structural, physiological, catalytic and regulatory (Underwood and Suttle 2004).

Numerous studies aimed to investigate factors that affect the absorption and metabolism of the minerals, as interaction with other nutritional or environmental factors seem to affect the adequacy or excess of these elements in the organism and to change the organism requirements (Underwood and Suttle 2004; Radostits et al. 2008). So, gastrointestinal nematode parasitism (GIP) is an important factor to be investigated for its effect upon macro- and micro-mineral metabolism. Studies to date in sheep are controversial for many of these parameters, as well as for the implicated mechanisms (Poppi et al. 1985; Bown et al. 1989; Coop and Holmes 1996; Underwood and Suttle 2004).

The present study aimed to investigate the concurrent effect of experimental gastrointestinal infection upon serum calcium, phosphate, magnesium, potassium, sodium, copper and iron concentrations in growing lambs during a 2-month period.

Materials and methods

Animals and management

The study was carried out using blood samples from lambs used in the experiment described in detail by Deligiannis et al. (2005). For this experiment were used 12 entire male lambs of the Karagouniko breed for a period of 8 weeks. All lambs were housed in individual pens, in a sheep shed that was bedded with straw and ventilated naturally. Each individual pen measured 1 × 1.50 m and was equipped with feed boxes for concentrate and hay, as well as a plastic trough providing a constant supply of fresh water. Before the start of the experiment, the lambs had been vaccinated against clostridiosis and they were de-wormed with a single dose of albendazole (7.5 mg/kg; Albendazole, Veterin®, S.A.). All lambs were free from trematodes, tapeworms and coccidia.

Lambs had ad libitum access to a pelleted concentrate mixture that was formulated according NRC (1985). The diets were formulated to satisfy or exceed NRC (1985) nutrient requirements of sheep. The concentrate mixture was composed of 450 g yellow corn, 250 g barley, 175 g cottonseed meal, 100 g soybean meal, and 25 g mineral and vitamin premix per kg. It contained 11MJ ME and 191 g crude protein/kg DM. In addition to the pelleted concentrate, each lamb received a daily allowance of about 100 g of un-chopped lucerne hay, containing 162 g CP/kg DM, 7.8 MJ ME/kg DM, and 290 g crude fiber/kg DM, to ensure normal rumen function. The lambs were given free and continuous access to fresh water.

Experimental design

After an acclimatization period of 21 days, the lambs were randomly divided into 2 groups (Group 1 and Group 2), that consisted of 6 animals each and the only criterion taken into account was the body weight (24 ± 1.6). Lambs of the Group 1 were infected with a single dose of 15,000 L_3 larvae of GI nematodes (3 ml × 5000 L3 larvae of *Trichostrongylus* 43.3%, *Teladorsagia* 23.3%, *Haemonchus* 20.0%, *Cooperia* 10.0% and *Oesophagostomum–Bunostomum* 3.4%). The L_3 larvae originated from coprocultures of faeces obtained from sheep naturally infected with a mixture of nematodes. The *Trichostrongylus* species used were: *T. colubriformis*, *T. axei* and *T. vitrinus.* Animals of the Group 2 remained uninfected and served as controls.

Clinical examination, blood sampling, analytical techniques and parasitological examinations

All lambs used in this study were clinically examined before and over the time of the experiment according to Radostits et al. (2008).

Blood samples were collected from each individual animal in all the experimental treatments at four time points (14, 28, 42 and 56 days after the infection with GI nematodes). The blood was obtained by jugular vein puncture in a vacuum glass tube using a tube holder with 20-gauge needle. After the clotting of blood, the tubes were then centrifuged at 3000 rpm for 15 min for serum separation. The separated serum was transferred into plastic vials and stored in a deep freezer for further biochemical analysis.

At the analyses, the serum samples were examined for mineral elements and particularly for calcium, phosphorus, magnesium, sodium, potassium, copper, and iron. The measurements of these elements were determined by means of atomic absorption spectrophotometry, using a Perkin-Elmer A Analyst 100 instrument, according to Perkin-Elmer analytical methods (1996).

Fecal samples were collected every week after infection directly from the rectum of each animal and were examined with the modified Mc Master technique (MAFF 1986).

Statistical analysis

The data were analyzed using SAS program (SAS Institute 2002). The results of mineral concentrations in serum were analyzed, at each time point after infection, using *t*-test to determine the difference between both uninfected and infected groups. Differences were considered to be significant at $P < 0.05$. Values are presented as arithmetical means with standard deviation (Mean ± SD).

Results

The lambs of the two groups did not show any clinical signs or abnormalities throughout the experiment. Faecal egg counts of the infected group at all the duration of the experiment were found as described/noted in Table 1.

Calcium, magnesium, phosphate, potassium and sodium concentrations in blood serum were not affected significantly from the parasitic infection (Table 2), as no statistical differences ($P > 0.05$) were observed among the experimental groups.

Serum copper concentrations were significantly lower ($P < 0.05$) in GIP infected lambs (Group 1) compared with the non-infected ones (Group 2) regardless the time points (Table 2). Serum iron concentrations were not found

Table 1 Fecal egg counts of the infected group throughout the experiment

Days	Fecal egg count/g
14	0
28	1600
42	2300
56	2600

Table 2 Serum mineral concentrations in growing lambs infected or not with gastrointestinal nematodes at different time points

Days after infection	Group	Ca (mg/100 ml)	Mg (mg/100 ml)	P (mg/100 ml)	K (meq/l)	Na (meq/l)	Cu (μg/100 ml)	Fe (μg/100 ml)
14	G_1	11.57 ± 1.68	5 ± 0.23	9.90 ± 0.84	4.64 ± 0.27	154.50 ± 7.82	121 ± 20.93	294 ± 196.24
	G_2	12.74 ± 0.54	4.94 ± 0.13	11.45 ± 0.64	4.76 ± 0.27	153.60 ± 7.23	78 ± 14.14	313.67 ± 144.79
28	G_1	12.55 ± 1.74	4.18 ± 0.32	10.82 ± 1.48	5.17 ± 0.25	146 ± 5.62	116.50 ± 15.26	313.50 ± 25.37
	G_2	12.20 ± 1.58	3.68 ± 0.22	10.42 ± 1.31	4.72 ± 0.26	148.80 ± 6.38	89.50 ± 20.73	250 ± 43.84
42	G_1	9.87 ± 1.70	4.32 ± 0.62	8.95 ± 0.48	5.28 ± 0.33	155.83 ± 2.99	131.50 ± 23.36	319 ± 79.83
	G_2	11.35 ± 2.43	3.13 ± 0.32	9 ± 1.59	5.22 ± 0.41	153.83 ± 5.95	84.50 ± 30.47	298 ± 60.59
56	G_1	11.98 ± 2.74	3.08 ± 0.32	8.53 ± 0.60	4.37 ± 0.24	151.80 ± 8.38	151.80 ± 8.38	312.83 ± 101.92
	G_2	10.57 ± 3.01	2.78 ± 0.33	10.82 ± 2.28	4.18 ± 0.59	152.60 ± 10.11	152.60 ± 10.11	290.50 ± 50.24

significantly different ($P > 0.05$) between the 2 groups at all the samplings.

Discussion

The present study aimed to investigate the effect of the experimental infection with gastrointestinal nematodes (GIN) upon the serum concentrations of calcium, magnesium, phosphate, potassium, sodium, copper and iron in growing lambs. According to the best knowledge of the authors, a similar study has not been conducted to date.

The range of serum calcium levels in all lambs of the present experiment were within the normal ranges that recorded by Radostits et al. (2008). Parasitic infection did not affect significantly the calcium serum concentrations in the present study. In previous studies the results seem controversial, as gastrointestinal parasitism was found to decrease the serum calcium concentrations in sheep significantly (Thamsborg and Hauge 2001) or not (Bown et al. 1989). Similar findings were observed in the present study for the experimental parasitism. Surprisingly, in an experimental infection with *Trichostrongylus rugatus* of lambs aged 5 months old Ca concentration increased in the infected group, without the authors to give a convincing explanation for it (Pullman et al. 1989).

Serum magnesium concentrations were not significantly different among lambs infected or not with GI nematodes. Similar results have been found in sheep with gastrointestinal parasitism (Bown et al. 1989; Thamsborg and Hauge 2001). It is worth mentioning, that the concentrations of serum magnesium levels were gradually decreased over the time of experiment in all animal groups, but still within the respective reference ranges of sheep. It is known, that the intestinal absorption efficiency of magnesium is markedly decreased in young ruminants (Radostits et al. 2008).

The obtained results revealed that the serum phosphate levels were not significantly different between animal groups over the time of experiment. In other similar studies, long time infections with *Trichostrongylus rugatus* (Pullman et al. 1989), *T. colubriformis* sole (Poppi et al. 1985) or together with *O. circumcincta* (Bown et al. 1989) led to decreased phosphate concentrations in blood serum. However, mid to heavy natural infections with *Ostertagia* spp. and *Trichostrongylus* spp. did not affect the serum phosphate concentrations in grazing lambs (Thamsborg and Hauge 2001) in Denmark. In the present study results regarding phosphate are similar with the results of Thamsborg and Hauge (2001), possibly because the parasitic infection of our study was given only once and not continuously, as in the other experimental studies. So, possibly the infection status of the present study was similar with the natural infection in Denmark (Thamsborg and Hauge 2001).

The nutritional problems from potassium in nature usually come from its excess rather than from its deficiency (Unerwood and Suttle 2004). The only previous study that was found in the literature did not find any correlation between serum potassium concentration and gastrointestinal parasitism (Zajícek et al. 1978), as well as the results of the present study.

Sodium is the electrolyte that is mostly intimately associated with water balance. Most sodium disturbances tend to be primarily fluid problems, while the normal plasma concentration is around 135–155 mmol/l. The levels of serum sodium in all lamb groups over the time of experiment were within the respective reference ranges of serum sodium in sheep (Kaneko et al. 2008; Radostits et al. 2008). Serum sodium concentration fluctuations throughout the experiment were not affected by gastrointestinal parasitism, as also previous study shows (Zajícek et al. 1978).

Although many studies have been conducted to assess the effect of copper based chemical compounds on gastrointestinal parasitism (Fausto et al. 2014; Leal et al. 2014; Grosskopf et al. 2017), few studies have been conducted to assess the effect of gastrointestinal parasitism upon copper status of sheep. Serum copper concentrations in all lamb groups over the time of the experiment were within respective reference ranges described by Radostits et al. (2008). Gastrointestinal parasitism reduced significantly the serum copper concentrations. This finding is supported also by other studies (Hucker and Yong 1986; Bang et al. 1990; Poppi et al. 1990; Kozat et al. 2006).

A decrease of whole blood copper levels has been demonstrated in sheep infected with Trichostrongylus spp. (Frandsen 1982) and with Haemonchus spp. (Hucker and Yong 1986). The GIN infections in sheep interfere with copper metabolism by causing an increase in pH of abomasal and duodenal digesta (Bang et al. 1990). Frandsen (1982) and Ortolani et al. (1993) showed that plasma copper was decreased by 50% in lambs challenged with infective larvae of *H. contortus*. According to Ivan (1988), availability of copper in sheep was decreased by helminthiasis and the magnitude of the effect was independent of copper concentrations in the diet.

However, it is considered that the mechanism of parasitism action upon copper serum concentrations may not be entirely by blocking the absorption from gastrointestinal tract, as copper serum concentration is affected also if copper is administered parenterally in sheep with GIN (Adogwa et al. 2005).

Serum iron concentrations were within the normal limits throughout the experiment (Radostits et al. 2008). Gastrointestinal parasitism reduced serum iron concentrations throughout the experiment, but the reduction was insignificant. It is known that parasitic infestation with severe blood loss can cause iron-deficiency anemia (Underwood and Suttle 2004; Adogwa et al. 2005). In other studies, there was found a decrease in serum iron concentration of naturally infected sheep (Kozat et al. 2006; Pandit et al. (2010). In the present study possibly the infective doses of parasites such as *Haemonchus* spp. and *Teladorsagia* spp. that can cause iron-deficiency anemia were low or the duration of the experiment was not long enough, to decline serum iron concentration significantly.

Authors contribution NG contributed in the chemical analyses and writing. MMA contributed in the chemical analyses and writing. IY contributed to statistics and writing. HB contributed to statistics and writing. EP contributed to parasitological study and writing.

Compliance with ethical standards

Ethical standards All experimental procedures in this study were carried out in compliance with the national guidelines and ethics and approved by the Veterinary Authorities (Magnisia Municipality, 2102/16-05-2002). All experimental lambs belonged to the local flock of the National Agricultural Research Foundation (NARF) station and consent to participation was obtained.

Conflict of interest The author declares that they have no conflict of interest

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