

A STUDY ON SOME BIOCHEMICAL PARAMETERS AND BLOOD MINERALS IN LAMBS WITH EXPERIMENTALLY INDUCED *HAEMONCHUS CONTORTUS* INFECTION

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ABSTRACT

This study was focused on changes of some minerals and blood metabolites during experimentally induced *H. contortus* infection in lambs. A total of 12 animals were included in the study and were divided into experimental (n=6) and control (n=6) groups. The lambs from experimental group were orally inoculated with 4000 infective *H. contortus* larvae (L3) by placing a probe. Blood parameters (calcium, phosphorus, magnesium, creatinine, glucose, total bilirubin, urea and alkaline phosphatase) were monitored on post infection days 0 and 21. The most pronounced changes occurred in urea and alkaline phosphatase levels, which increased significantly on day 21 ($p < 0.05$) and reached values of 12.17 ± 0.24 mmol/L and 334.17 ± 32.3 U/L, respectively. The concentration of urea in experimental group was higher by 46.82% compared to control group. Alkaline phosphatase activity was also increased by 55.07% vs. control group. Mild changes occurred in plasma concentrations of calcium, phosphorus, magnesium, creatinine, glucose and total bilirubin but were not considered significant throughout the experiment.

Key words: *Haemonchus contortus*, lambs, blood minerals, biochemical parameters.

Introduction

Sheep farming is a widespread livestock husbandry branch in many countries around the world including Bulgaria. The lack of mandatory systemic prophylaxis against parasites is responsible for frequently occurred infections with gastrointestinal helminths in sheep. *Haemonchus contortus* (Nematoda, Trichostrongylidae) is outlined as one of most pathogenic members of this family causing serious economic losses at a global scale. Data concerning the influence of *H. contortus* on host mineral metabolism are limited and often contradictory. Georgieva et al. (1975) observed decreased levels of calcium, phosphorus and partially magnesium in experimentally infected with *H. contortus* sheep. Comparable investigation on sheep haemonchosis disclosed hyperphosphatemia and hypocalcemia (Gilani, 1981). According to Abakar et al. (1999-2000), inorganic phosphorus showed no alterations while the levels of blood calcium were decreased. In contrast, Nematollahi et al. (2004) reported no deviations in the quantity of phosphorus and magnesium levels in blood serum during *H. contortus* infection in sheep. In addition, *H. contortus* are also capable of inducing heavy alterations of host serum enzyme system. Changes of alkaline phosphatase activity in sheep and goats could be significantly elevated (Ahmad et al., 1989; Bahrami et al., 2011; Marume et al. 2012) as well as the reference values have been also observed throughout the course of *H. contortus* infection (Nematollahi et al., 2004). Data concerning the influence of *H. contortus* on quantity of some metabolic waste are limited or completely lacking. Min et al. (2015) reported no alterations in

plasma concentration of total bilirubin, glucose and creatinine during haemonchosis in goats. The increased urea levels have been reported in experimentally infected lambs (Khan et al., 2012).

Materials and methods

Inoculum preparation

A sheep with pure *H. contortus* infection served as a donor for harvesting eggs from faeces. The stool samples were obtained manually from *ampulla recti*, placed in petri dishes and incubated at 27°C for a week. Infective *H. contortus* larvae (L₃) were collected by larval migration technique according to Baermann.

Experimental animals

An approval for using animals in experiment was obtained from the Bulgarian Food Safety Agency. The study was performed with 12 three-month-old lambs divided into experimental (G1; n=6) and control (G2; n=6) groups. The animals from G1 were orally inoculated with 4000 *H. contortus* L₃ by placing a probe.

Sampling and assaying

Blood was collected by venepuncture of *v. jugularis externa* from each animal into sterile tubes containing anticoagulant (K₂ EDTA). Plasma samples were obtained by centrifugation at 500 rpm for 10 minutes and were used immediately for quantitative determination of calcium, phosphorus, magnesium, creatinine, glucose, total bilirubin, urea and alkaline phosphatase on post infection days 0 and 21. Samples were assayed by automated biochemical analyzer Mindray BS – 120 (Sweden).

Statistical analysis

All data were analyzed by means of MedCalc v.10.2.0.0, MedCalc Software (Belgium). The differences between experimental and control animals were compared by unpaired t-test and were considered significant at $p \leq 0.05$. The obtained values are represented as mean $\pm$ standard error of mean (SE $\bar{x}$).

Results and discussion

Mineral elements

The obtained values apparently indicate that no disturbances occur in the plasma levels of calcium, inorganic phosphorus and magnesium, which remained stable, and within the reference ranges during the study period. However, the mild alteration was recorded only in calcium level that dropped by 7.2% compared to the baseline but it was considered not significant. Similarly, inorganic phosphorus and magnesium remained unaffected throughout the trial period. The comparable investigations revealed a significant reduction of calcium and phosphorus blood levels in lambs on the 30th and 40th day post inoculation, respectively (Georgieva et al., 1975). The same research team also reported no alterations of magnesium metabolism during the course of *H. contortus* infection. The lack of changes in our study is probably due to the short trial period. Similar investigation carried out by Nematollahi et al. (2004) demonstrated no abnormalities in phosphorus and magnesium levels in sheep haemonchosis, which is in general agreement with our conclusion. Abakar et al. (1999-2000) have not been observed the connection between phosphorus concentrations and *H. contortus* infection in sheep. Conversely, Gilani (1981) reported hyperphosphatemia after conducting the similar trial. The contradictions between our results and the

data presented by cited authors are most likely due to differences in administered dose infective larvae, which plays a key role in pathogenesis of haemonchosis including mineral metabolism. In addition, the content of mineral substances in the feed also influences the quantitative changes in serum calcium, phosphorus and magnesium levels in blood.

Creatinine, blood glucose, total bilirubin, urea, alkaline phosphatase

Creatinine and total bilirubin were slightly altered but the changes were within the reference intervals. The blood glucose level remained unaffected in G1 group at the end of the trial (Table 1). Similar statement was reported by Min et al. (2015) in experimentally induced haemonchosis in goats. The blood glucose level also remained unaffected during the experiment. Other investigation disclosed that *H. contortus* infection has a marked impact on glucose concentration in the blood of host (Thivend, 1974; Ward, 1974). The same researchers assumed that both tissue larvae and adults *H. contortus* use the glucose as the main energy source. Available data demonstrated a significant decline of blood glucose in sheep infected with gastro-intestinal strongylids (Kouider et al., 1994). However, hypoglycemia could not occur even in severe infections (Siddiqui et al., 2005). In this connection, the glucose homeostasis has not been affected in sheep acquired the intestinal strongylid *Trichostrongylus colubriformis* (Roseby, 1973).

Table 1. Changes in biochemical parameters (mean $\pm$ SE_s) in lambs with experimentally induced *H. contortus* infection

	Day 0	Day 21
Calcium (mmol/L)	3.21 $\pm$ 0.09	2.98 $\pm$ 0.11
Phosphorus (mmol/L)	2.05 $\pm$ 0.14	1.71 $\pm$ 0.22
Magnesium (mmol/L)	0.9 $\pm$ 0.03	0.86 $\pm$ 0.03
Creatinine (μ mol/L)	84.67 $\pm$ 3.5	88.83 $\pm$ 2.18
Total bilirubin (μ mol/L)	4.85 $\pm$ 0.38	5.87 $\pm$ 0.29
Glucose (mmol/L)	3.91 $\pm$ 0.25	4.04 $\pm$ 0.08
Urea (mmol/L)	9.63 $\pm$ 0.84	12.17 $\pm$ 0.24*
Alkaline phosphatase (U/l)	243.3 $\pm$ 23.3	334.17 $\pm$ 32.3*

Urea, alkaline phosphatase

The most pronounced changes occurred in the levels of urea and alkaline phosphatase. The quantity of blood urea was elevated by 46.82% vs. G2 and by 26.38% vs. initial value. Both differences were considered significant ($p \leq 0.05$). Alkaline phosphatase activity was also significantly increased by 55.07% vs. G2, and by 37.35% vs. baseline (Table 1). The same conclusion was reported by Khan et al. (2012). According to Angulo-Cubillan et al. (2007), the helminthes excrete ammonia radicals, which are toxic and undergo detoxification in the host liver resulting in urea synthesis that passes into the blood. Our results also revealed a significant increase in alkaline phosphatase in infected lambs. Similar data have been reported in sheep and goats with natural or experimentally induced *H. contortus* infection (Ahmad et al., 1989; Sharma et al., 2001; Bahrami et al., 2011; Roy et al., 2013).

Conclusion

H. contortus is considered as one of the most distributed nematode species in sheep with heterogeneous pathogenic effect on the host. Our study was focused on performing the experimentally induced moderate infection in lambs that act as specific hosts. It was confirmed that *H. contortus* strongly affects mainly the elements of circulatory system of the host. Data concerning the influence of this parasite on mineral metabolism as well as levels of some metabolic waste is limited or completely lacking. The results obtained here demonstrated that *H. contortus* does not cause severe damages on host mineral homeostasis (calcium, phosphorus and magnesium) but could

be involved as a secondary factor inducing accumulation of some metabolic waste (urea) in the blood. It was very likely due to its own toxins secreted throughout the prepatent period.

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