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Original Contribution

A STUDY ON PLASMA FIBRINOGEN AND HAPTOGLOBIN IN LAMBS WITH EXPERIMENTALLY INDUCED *HAEMONCHUS CONTORTUS* INFECTION

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ABSTRACT
The present study aims to evaluate the changes in concentrations of some acute phase proteins during *Haemonchus contortus* infection in lambs. This experiment was performed using 12 three-month-old healthy lambs, randomly allocated into 2 equal groups: G1 (uninfected control animals) and G2 (lambs infected with *H. contortus*). Each lamb of G2 group was orally inoculated with 4000 infective third stage larvae (L_3) of *H. contortus* by placing a probe. Blood samples were collected on post infection days zero, 4, 7, 11, 14, 21, 28 and 32. After sampling, the blood was centrifuged, and the separated plasma was used for the quantitative determination of haptoglobin (Hp) and fibrinogen (Fb). The most pronounced changes occurred in Hp levels, which increased and attained the highest values on post infection days 4, 7, and 11. A peak concentration occurred on post infection day 7 when Hp increased by 45.96% vs the initial level, and by 44.08% vs the control level on the same day. There were no significant changes in Fb concentrations throughout the study.

Key words: Acute phase proteins, haptoglobin, fibrinogen, *Haemonchus contortus*, sheep

INTRODUCTION

Haemonchus contortus (Strongylida: Trichostrongylidae) is one of the most common nematodes affecting small ruminants, primarily in countries with warmer and humid climate, including Bulgaria (1, 2). Throughout the life cycle, both adult parasites and tissue larvae fourth stage (L_4) have direct and indirect effects on the host, causing a variety of local and systemic responses. In this regards, *H. contortus* infection entails severe disturbances in the integrity of the abomasal mucosa (1) inducing an acute phase response (APR). This early defensive systemic reaction is activated by different pathological processes e.g. trauma, surgery, viral, bacterial and parasitic infections, etc. (3). Tissue integrity damage is the primary stimulus activating different classes of white blood cells to synthesize IL-1, IL-6 and TNF-α (4). Those pro-inflammatory cytokines trigger a series of biochemical events in hepatocytes, manifested with enhanced production of acute phase proteins (APPs) (5). Depending on the quantitative changes during APR, APPs are divided into three groups - major, moderate and minor APPs. In small ruminants, Hp is considered as a major APP, whereas Fb is classified as a minor APP (6). Despite the potential value of APPs as biomarkers of infection to monitor the health status of the animals, information about Hp and Fb levels in sheep with parasitic diseases is limited. Given the above, the present study aimed

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to investigate Hp and Fb changes in sheep with experimentally induced *H. contortus* infection along a time course of 32 days, to provide a background for early diagnosis.

MATERIAL AND METHODS

Experimental animals

Prior to the study, an approval for using animals in an experiment was obtained from the Bulgarian Food Safety Agency (permit №107/2014). A total of 12 three-month-old heathy lambs were included in the experiment. The animals were randomly allocated into 2 equal groups – G1 (uninfected control animals) and G2 (infected animals). Infected lambs were reared separately in the isolation unit of the Infectious and Parasitic Diseases Ward at the Faculty of Veterinary Medicine, Stara Zagora. After housing in the premises, the animals were allowed a 3-week period of adaptation. Before the infection, all lambs were examined for presence of helminthic and protozoan infections through routine ovascopic and larvascopic laboratory techniques. Each lamb of G2 group was orally infected with 4000 L_3 of *H. contortus* by placing a probe.

Sampling and assaying

Blood samples were collected by venipuncture of *v. jugularis externa* into heparin tubes on the zero, 4^{th}, 7^{th}, 11^{th}, 14^{th}, 21^{st}, 28^{th} and 32^{nd} day post-infection (DPI) of the animals. After sampling, the blood was centrifuged at 2000 rpm for 10 min, and the separated plasma was used for quantitative determination of Hp (hemoglobin-binding colourimetric assay, Tridelta Ltd., Ireland) and Fb (nephelometric method of Podmore) (7).

Statistical analysis

The data were analyzed by means of one-way ANOVA (Statistics for Windows, Stat Soft.). Obtained values were expressed as mean ± standard deviation (SD), and the differences were considered significant at $P<0.05$.

RESULTS

As shown in **Table 1 and Figure 1**, plasma Fb level was not changed throughout the entire experimental period, and its values showed weak fluctuations in both groups. However, Fb levels in the experimental group were higher than those of controls. This was more obvious on post infection days 11, 21, 28 and 32. In contrast, Hp concentrations were significantly increased in G2 group on the 4^{th} (552±34.4 mg/mL), 7^{th} (658±42.5 mg/mL) and 14^{th} (611±41.9 mg/mL) DPI (**Table 1 and Figure 2**). The peak level occurred on the 7^{th} DPI when its concentration increased by 45.96% vs initial level and by 44.08% vs control level on the same day. In addition, there were significant differences in G2 compared to initial values on the 7^{th} and 14^{th} DPI.

Table 1. *Plasma concentrations of Hp and Fb in lambs infected with H. contortus*

Parameter	Group	Time							
		Day 0	Day 4	Day 7	Day 11	Day 14	Day 21	Day 28	Day 32
Hp (µg/mL)	G1	390.7±33.4	362.7±30.1	456.7±39.6	417.5±30.5	460.7±38.7	455±13.5	397.2±15.0	382.2±17.7
	G2	450.8±46.8	552±34.4^{a1}	658±42.5^{a2b2}	419.3±43.1	611±41.9^{a1b1}	428.2±30.9	414.5±28.8	419.5±22.1
Fb (g/L)	G1	2.75±0.16	2.96±0.22	2.55±0.29	2.22±0.34	2.50±0.31	2.40±0.42	2.33±0.24	2.33±0.21
	G2	3.19±0.27	3.05±0.18	2.61±0.22	2.76±0.23	2.55±0.20	2.82±0.29	2.97±0.27	2.76±0.17

Significance between groups: $^1p < 0.05$; $^2p \leq 0.01$

Significance in the G2 to the initial level: $^ap < 0.05$; $^bp \leq 0.01$

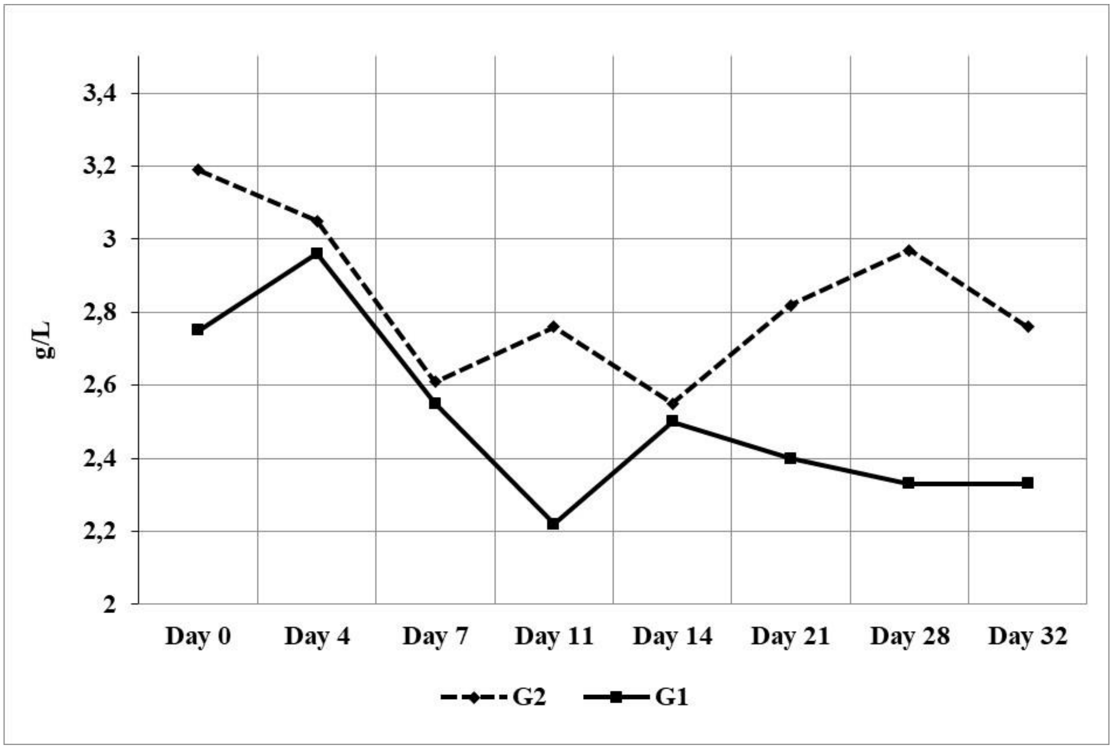

Figure 1. Plasma concentration of Fb in lambs infected with *H. contortus*
Significance between groups: [1]$p < 0.05$; [2]$p \leq 0.01$
Significance in the G2 to the initial level: [a]$p < 0.05$; [b]$p \leq 0.01$

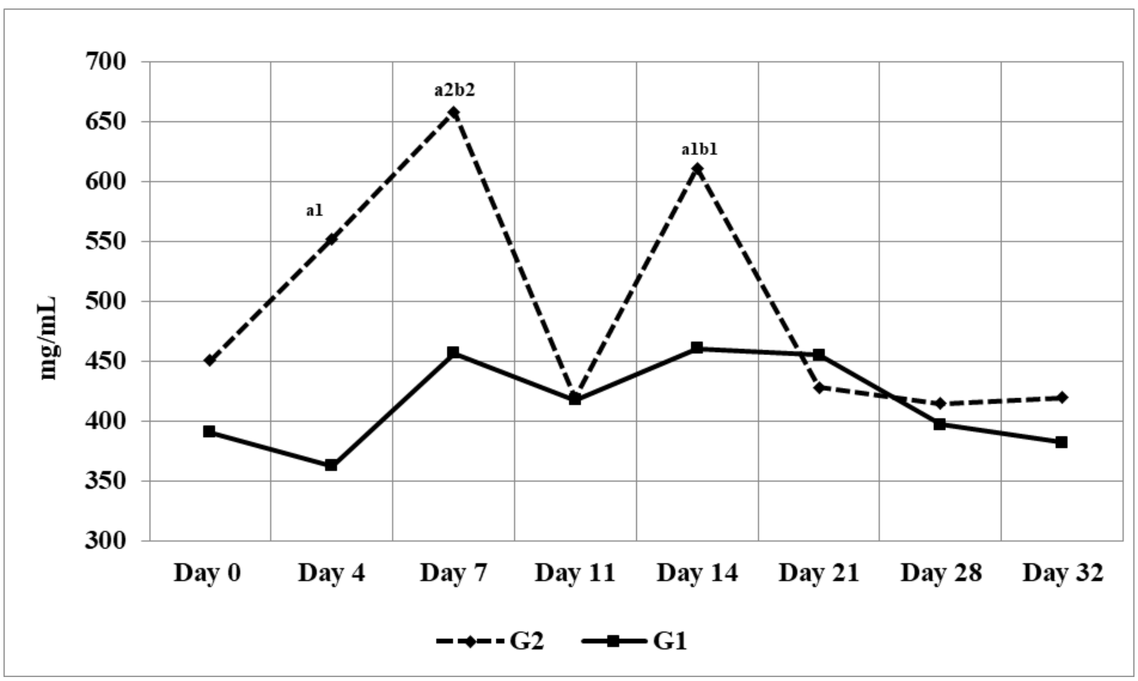

Figure 2. Plasma concentration of Hp in lambs infected with *H. contortus*
Significance between groups: [1]$p < 0.05$; [2]$p \leq 0.01$
Significance in the G2 to the initial level: [a]$p < 0.05$; [b]$p \leq 0.01$

DISCUSSION

This study measured Hp and Fb concentrations after experimentally induced infection with *H. contortus* in order to find an indication for early diagnosis of haemonchosis in sheep. The obtained data demonstrated that *H. contortus* had a marked effect on plasma Hp levels. As mentioned above, Fb levels were relatively constant, although they were higher in the infected group when compared to the control animals. This increase was probably due to the sexual maturation of *H. contortus* which is in line with statement of Soulsby (8). The author affirms that tissue L_{4s} induce substantial injuries of the abomasal mucosa in earlier stages of the infection. The later increase in Fb levels is likely due to mature parasites that often change their attachment site on the abomasal mucosa and induce bleeding lesions (9). In addition, adult worms cause blood coagulation inhibition (10) and Fb does not convert to fibrin, interrupting the feedback for its liver synthesis and hence, its plasma concentrations remained normal. The minor response of Fb could be also attributed to some specific feature of ovine APR. According to Gomez-Laguna et al. (6), Fb belongs to the group of minor APPs in sheep and its response to tissue damage is usually weaker. In contrast, Hp is the most specific reactant in small ruminants. In our study, plasma Hp increased considerably as early as the beginning of the experimental infection (day 4) and peaked on the 7^{th} and the 14^{th} DPI when its values were almost doubled. It is known that the first response to tissue damage is that of the non-specific systemic defense, including APR (11, 12, 13). Authors reported that in the early acute stage of inflammation, the concentration of APPs increased rapidly during the first few hours after the challenge, and peaks occur within 24-48 hours. Nevertheless, this time course is not obligatory and depends both on the pathogen and host species. The early response of Hp in current study matches the occurrence of L_{4s} in the abomasal mucosa, associated with mechanical damage and impaired integrity. Detected peaks on 7^{th} and 14^{th} DPI probably corresponded to the growth of larvae and more severe mechanical damage of the mucosa. These findings are in general agreement with the results of Zhong et al. (14) regarding the APR of sheep infected with *H. contortus*, which exhibited the strongest increase in plasma Hp on 3^{rd} and 28^{th} DPI. Increased Hp level is also reported by Ulutaş et al. (15) in goats infected with trichostrongylides, *Trichuris* spp. and *Fasciola* spp. To the best of our knowledge, there are no other data on the levels of APPs in sheep infected with *H. contortus*.

CONCLUSION

This study investigated some plasma APPs in lambs with experimentally induced haemonchosis, presenting evidence that Hp, could be useful as an auxiliary biochemical indicator for diagnosis and monitoring purposes for this disease. In contrast, the Fb level is not specific marker for ovine haemonchosis.

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