

## Evaluation of Total Protein, Albumin, Globulin and Blood Urea Nitrogen Concentrations in Gastrointestinal Nematodes Infected Sheep

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**Abstract:** Gastrointestinal parasites (GIP) are one of the most important impacting factors on animal breeding management and have negative effect on protein metabolism in ruminant. The main objective of this study was to evaluate the change of some plasma biochemical parameters related to protein metabolism such as plasma total protein, albumin, globulin and blood urea nitrogen concentrations and albumin: globulin ratio during naturally acquired infection with gastrointestinal nematodes in sheep. In this experiment, a total of 35 non pregnant, aged 6 to 12 months and  $29 \pm 2$  kg the average of body weight were selected among the naturally acquired nematode infected animals which their infection had confirmed by McMaster flotation method. The animals were divided into 3 groups according to the faecal egg counts (FECs) (< 350, 350 – 450 and > 450). Blood samples were taken from the jugular vein of animals after 7 hours fasting period and faecal samples were taken from the rectum of each animal, concurrently. All the animals had not received any anthelmintic drugs at least 4 months prior to sampling. The plasma was analyzed to determine of albumin: globulin ratio and total protein, albumin, globulin and blood urea nitrogen concentrations by spectrophotometric method using the commercial kits. Our results indicated that there was no a significant difference among groups concerning all the studied parameters. Also, there was a positive significant correlation between EPGs and albumin concentration ( $P < 0.05$ ,  $r = -0.407$ ). In conclusion, the load of GIN infection (EPGs < 850) had no significant effect on plasma total protein, albumin, globulin and blood urea nitrogen concentrations and albumin: globulin ratio during naturally acquired infection with gastrointestinal nematodes in ewes. With an increase in load of GIN infection, the concentrations of total protein and albumin were decreased and the concentrations of globulin and BUN were increased, non-significantly.

**Key words:** Faecal egg counts • Biochemical parameters • Plasma • Sheep

### INTRODUCTION

Small ruminants are reared mainly for milk, meat, hides and wool production in various regions of the world. Farmers are trying to develop appropriate management methods to reduce the cost and raise the quality of products which they introduce to customer. Overall, nematodes are the most prevalent GIP in the world [1-4]. In most countries, small ruminants are fed at pastures where there was not a good predictive and hygienic design to control parasites; therefore, the most common diseases in small ruminants are GIP infections which affect the performance of infected animals. Nematodes have

relatively simple structure, cylindrical and non-segmented bodies which make their diagnosis and identification be easier compared to other helminthes. They have various life cycles and may be found in water, soil, animals and other organisms during the different stages of their life cycle; therefore, nematodes have a world distribution. The common GIPs of livestock include: *Trichostrongylus*, *Ostertagia*, *Haemonchus*, *Nematodirus* and *Cooperia* genus [5-7]. Gastrointestinal parasites cause development of immature cells which lead to formation of weak cell junctions. Therefore, macromolecules such as proteins enter the abomasums and intestine through the mucosa [5, 8]. The protein excretion into the intestine causes a

decrease of blood total protein concentration, particularly albumin, which has an impacting effect on protein metabolism in livestock [9]. The plasma total protein is mainly composed from albumin (about 35 – 50%) in animals and this protein acts as a carrier for transport of different components such as hormones (e.g. thyroxine), fatty acids and amino acids. Additionally, albumin is as a large amino acid and proteins reservoir in body [10, 11]. The low level of protein caused reduction in ruminant's prolificacy, growth rate, wool and milk production and reproductive performance [12]. Moreover, the rate of urea synthesis increases in the GIP infections in ruminants, which is probably associated with increase of ammonia metabolism due to amino acids deamination [13, 14]. Therefore, the efficiency of protein absorption is necessary to yield high-quality products in small ruminants. The purpose of this study was to evaluate the change of A: G ratio and plasma total protein, albumin, globulin and blood urea nitrogen concentrations during infection with gastrointestinal nematodes in ewes.

## MATERIALS AND METHODS

**Animals and Experimental Design:** The experiment was performed at the Moghan plateau, Ardabil province, Northwest of Iran 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. Faecal samples were taken from the rectum of 350 Moghani ewes and kept in individual sampling containers under 4°C and all the fecal samples were monitored for finding the GIP infected animals. Their infection confirmed by McMaster flotation method. Among them, a total of 35 non-pregnant, 6-12 months old and 29±2 kg body weight of grazer Moghani ewes were randomly selected which their infection had confirmed previously. The animals were divided into three groups according to EPGs (<350, 350 - 450 and >450). During the study, all the animals fed *ad libitum* by grazing at Moghan plateau and had free access to water.

**Sampling Procedure:** Blood and faecal sampling was performed after 7 hours fasting period. Blood samples were collected from the jugular vein of all the ewes using disposable syringes containing heparin as an anticoagulant, then the samples were centrifuged at

750× g for 10 minute and plasma was separated and stored at -20 °c until used. Concurrently, faecal samples were taken from the rectum of each animal and kept in individual sampling containers under 4°C.

**Laboratory Analyses:** Faecal egg count was determined using McMaster flotation technique [15] at laboratory of parasitology at the Islamic Azad University, Shabestar branch. Shabestar, Iran. The majority sizes of the observed gastrointestinal eggs using scaled microscope were between 70 - 90 µm. These egg sizes were consistent with the nematodes eggs which were commonly found in most small ruminant (e.g. *Trichostrongylus*, *Ostertagia*, *Haemonchus* and *Nematodirus* genus). Plasma was used to determine total protein, albumin, globulin and blood urea nitrogen concentrations. All the studied parameters 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. Also, albumin: globulin ratio was calculated using excel software.

**Statistical Analyses:** 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 at the  $P < 0.05$  level between the means. All values are shown as mean ± standard deviation (SD).

## RESULTS

The mean ± standard deviation and 95% confidence interval of all the studied parameters in different groups are presented in tables 1 and 2, respectively. According to our results, there was non significant difference between groups concerning all the studied parameters ( $P > 0.05$ ). Additionally, there was not any overlap between groups concerning all the studied parameters. Correlation coefficients and statistical analysis between all the parameters in Moghani ewes are shown in table 3. We observed a negative significant correlation between EPGs and albumin concentration ( $P < 0.05$ ,  $r = -0.402$ ). As well as, our results cleared that there was a positive but not significant correlation between EPGs with globulin ( $r = 0.054$ ) and BUN ( $r = 0.260$ ) concentration ( $P > 0.05$ ). The mean of total EPGs of all the studied ewes was approximately 850.

Table 1: The values of studied plasma biochemical parameters in naturally nematode infected Moghani ewes

Group	EPGs	N		Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	BUN (mg/dl)	A:G
I	< 350	12	Mean	7.15	4.29	2.86	22.22	1.66
			SD	0.75	0.60	0.90	3.49	0.63
II	350 - 450	12	Mean	7.38	4.29	3.09	22.93	1.51
			SD	0.63	0.51	0.75	5.78	0.60
III	> 450	11	Mean	7.09	3.95	3.14	24.93	1.43
			SD	0.65	0.54	0.70	4.97	0.57

A: G albumin: globulin ratio; SD Standard deviation; EPGs egg count per gram of faeces; BUN blood urea nitrogen

Table 2: The values of 95% CI of studied plasma biochemical parameters in naturally nematode infected Moghani ewes

95% confidence interval								
Group	EPGs	N		Total Protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	BUN (mg/dl)	A:G
I	< 350	12		6.67-7.62	3.90-4.67	2.28-3.43	20.00-24.43	1.25-2.06
II	350-450	12		6.97-7.78	3.96-4.61	2.61-3.56	19.25-26.60	1.12-1.89
III	> 450	11		6.65-7.52	3.58-4.31	2.66-3.61	21.59-28.26	1.05-1.81

A: G albumin: globulin ratio; EPGs egg count per gram of faeces; BUN blood urea nitrogen; CI confidence interval

Table 3: Correlation coefficients and statistical analysis between EPGs and studied plasma biochemical parameters in all the naturally nematode infected Moghani ewes (n= 35)

		EPGs	Total protein	Albumin	Globulin	A:G	BUN
EPGs	Pearson Correlation	1	-0.272	-0.402*	0.054	-0.248	0.260
	Sig. (2-tailed)		0.114	0.017	0.758	0.150	0.132
Total protein	Pearson Correlation		1	0.220	0.711**	-0.424*	-0.022
	Sig. (2-tailed)			0.203	0.000	0.011	0.902
Albumin	Pearson Correlation			1	-0.529**	0.746**	0.059
	Sig. (2-tailed)				0.001	0.000	0.735
Globulin	Pearson Correlation				1	-0.906**	-0.063
	Sig. (2-tailed)					0.000	0.721
A: G	Pearson Correlation					1	0.014
	Sig. (2-tailed)						0.935
BUN	Pearson Correlation						1
	Sig. (2-tailed)						

A: G albumin: globulin ratio; EPGs egg count per gram of faeces; BUN blood urea nitrogen;

Correlation is significant at the 0.05 level (2-tailed). \*\* Correlation is significant at the 0.01 level (2-tailed).

## DISCUSSION

In the current study, the level of total protein was higher than the values reported previously in non - infected sheep [10, 16], infected goats [17] and sheep [18, 19] and also lower than the other reports in GIP infected goats [4, 20]. But, its value was consistent with the other values reported in sheep [21, 22]. Although, we showed a negative correlation between the EPGs and total protein concentration in infected Moghani ewes and the lowest concentration of total protein was in group III. The level of total protein is affected by various factors such as age, sex, species, breed, season, disease and it's during time (e.g. hepatic, renal and infections of different parasites), body hydration, nutritional and physiological status in livestock [4, 7, 10, 16-20]. During the GIP

infections a considerable quantities of the host proteins are lost into the parasites tract (approximately 10% total blood volume per day) [5, 12]. Concurrently, some of the proteins are lost through the faeces [23, 24]. Our results confirmed the negative effect of GIP on blood total protein concentration in ewes which were consistent with previous reports in sheep [7, 19]. The level of albumin was higher than the values reported in non-infected sheep [22] and infected goat with GIP [4, 17] and sheep [7, 19]. Also, the values of albumin in Moghani ewes were higher compared to the other breeds of Iranian sheep [10, 16]. Similar to total protein, the concentration of albumin was decreased with increases the load of GIN infection in ewes. Albumin is a negative acute phase protein and its concentration is decreased during inflammatory and parasitic infections [10, 11, 25]. Therefore, our result

seems normal. As well as, the GIP affects the feed digestion, energy and nitrogen utilization in parasitized animal and reduces the performance, feed intake and lead to hypoalbuminemia [5, 7, 26]. In this study, the concentration of globulin was higher than the values reported in other breeds of Iranian sheep [10] and infected goats [3, 17], but lower than the values reported in infected sheep [27]. Although, the concentration of globulin in infected Moghani ewes was consistent with the other values reported in sheep and cows [21]. Increases in load of GIP infection led to high globulin concentration in Moghani ewes which was consistent with other reports in sheep [27] and goats [17]. Globulin is the other part of total protein which is composed from various proteins, except to albumin and prealbumin. The members of globulin are mainly positive acute phase proteins, therefore, their concentrations increase during inflammatory and parasitic infections in livestock [10, 17, 22, 25]. The values of albumin: globulin ratio was higher compared to the other values reported in sheep [10, 21, 22] and infected goats [17]. Likely, addition to the load of infection and other aforementioned effecting factors which have complex effects on proteins concentrations, fasting may be a reason behind inconsistent results of the current experiment with other previous reports in small ruminants [10, 11]. Collectively, in this study, the globulin and albumin concentrations were changed; but as, albumin is the main blood protein and its variation is more predominant compared to globulin; therefore the changes of total protein concentration and albumin: globulin ratios in infected Moghani ewes were normal. As well as, in the current study, the concentration of BUN had a little difference with the other values reported previously in sheep [10] and higher than the values in sheep [16] and infected goat [4, 28]. But, its concentration was consistent with the other values reported in sheep [21, 22]. The change of BUN concentration was similar to the globulin in the infected Moghani ewes and its concentration was increased with the elevation of GIP load. Parasitized sheep had a higher plasma BUN concentration than non-parasitized animal which attributed to the rate of irreversible loss and the rate of urinary excretion of urea [13]. As well as, during the GIP infections, the level of plasma proteins are decreased and amino acids deamination is accelerated for proteins compensation which leads to increase BUN concentration in parasitized animal [14]. Our result concerning the change of BUN concentrations during the GIP infections agreed with the other previous reports in sheep [13, 14]. In the current

study, sex, season, nutrition and physiological state (pregnancy) had no effect on results, as the entire animals were reared and fed with the same status and had similar sexes and physiological status. In conclusion, the load of GIP had non significant effect on all the studied parameters. But, the load of GIP with the EPGs > 450 had more negative effect on total protein and albumin concentrations while had more positive effect on albumin: globulin ratio, BUN and globulin concentrations in Moghani ewes.

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