

From the State Veterinary Research Station for Small Ruminants,
Sandnes, Norway.

TOTAL SERUM PROTEIN LEVELS AND PAPER ELECTROPHORETIC PATTERNS IN PREGNANT EWES

By
Arne Hjelle

Serum protein studies in sheep by means of paper electrophoresis have been performed by many authors in both healthy and diseased animals (*Boguth 1954, Endrejat 1956, Jungmann 1960, Koenig et al. 1949, Perk & Lobl 1960 and Turner & Wilson 1962*). In healthy animals the electrophoretic pattern may vary depending on age, breed and nutritional state (*Vesselenovitch 1959, Ravaioli 1959*). As electrophoresis is a useful diagnostic tool, it is necessary to know, how these factors together with environmental and physiological conditions may influence the serum protein values in healthy animals. According to available literature no investigations seem to have been undertaken as to the influence of pregnancy on total serum proteins and their electrophoretic pattern in sheep. The investigations presented in this paper were carried out in order to record the above mentioned values during pregnancy in housed ewes under different feeding conditions and with informations of the nematode infestation based on egg counts.

MATERIALS AND METHODS

Serum samples from 48 pregnant ewes, 3—8 years old, all of the Norwegian Dala breed were examined. These ewes, divided into 4 groups of 12 each, were primarily part of a parasitological and haematological study of nematode infestation and anthelmintic treatment in cooperation with the Department of Internal Medicine, Veterinary College of Norway (*Helle et al. 1965*). The parasitological examinations were performed at the last-mentioned

laboratory. The results of these investigations, including haematological conditions, will later on be published in detail.

Total serum protein levels were determined by means of the Biuret method according to *Wootton* (1964) with monthly intervals from the 15th of December 1964 to the 15th of June 1965. As protein standard was used bovine serum albumin (Koch-Light Laboratories Ltd, England). Filter paper electrophoresis of the corresponding serum samples were performed using LKB-equipment with LKB-HR-buffer, pH 8.9 and ionic strength 0.6. The paper used was Schleicher & Schüll no. 2043. The samples were run for 18 hours with a current of 0.75 mamp. per paper at 125 v. The papers were stained with amidoblack, examined in Eel Scanner and calculated by planimeter. By this method 5 protein fractions were separated, viz. albumin, α_1 -, α_2 -, β - and γ -globulin. Due to unsatisfactory separations of α - and β -globulins in some samples the α_1 and α_2 -globulins were added and so were the β - and γ -globulins.

Nematode egg counts in faeces were made monthly from all groups from the 15th of December 1964 to the 15th of April 1965, after that time twice a month, using a modified Mc Master technic (*Helle* 1964). On the 18th of December 1964 group 1 was treated with Thiabendazole, on the 18th of March 1965 group 2 got the same treatment.

Groups 1—3 were kept on the same ration from the 15th of December 1964, i.e. hay and silage ad lib. and 150 g of concentrates per sheep per day. From the 12th of March 1965 the concentrates were increased to 300 g and 600 g, respectively. The concentrates used were a commercial mixture consisting of 18 % crude protein and 15 % digestible crude protein. The average time of lambing for all groups was on the 8th of April 1965, and the flock was moved out on pasture on the 23rd of April.

RESULTS

Fig. 1 shows the distribution of total serum proteins and protein components in absolute values during the period from December to June. Each group (columns 1, 2, 3 and 4) represents the average values of 12 animals, as there is no allowance made for the age of the animals. From December on there was a steady decrease in total protein content reaching the lowest values in April. There was one exception in group 1 as the total serum proteins increased at that time. In June the values of all groups

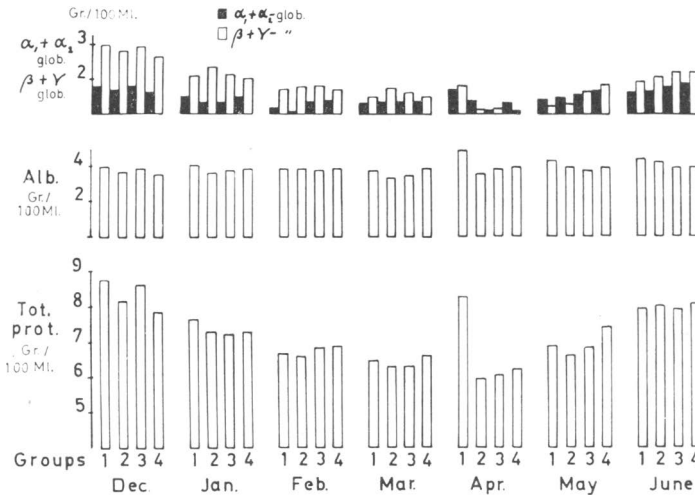


Figure 1. Distribution of total serum proteins and protein components.

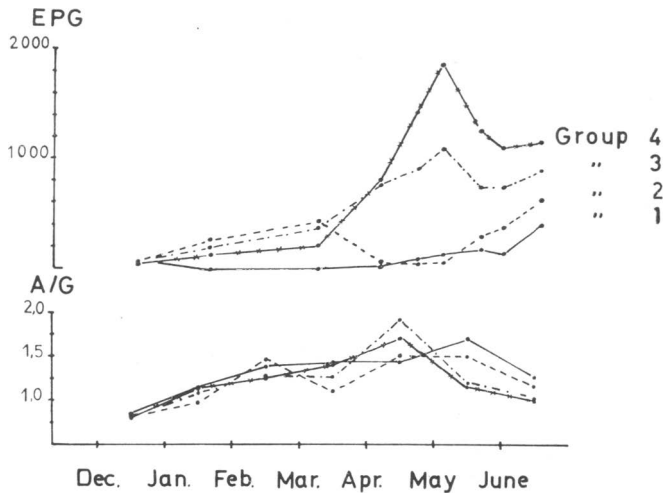


Figure 2. Albumin/globulin ratios (A/G) and faecal egg counts (EPG).

passed the level of January. The albumin values displayed great stability. Apart from group 1 the globulin values in April were low, in May, however, there was a distinct rise in these values following parturition and free access to pasture. The increase of total proteins in May in groups 3 and 4 was due to a rise in the globulin components, especially the γ -globulins in group 4. Fig. 2

compares the A/G ratio and EPG in the different groups from December to June. In December the A/G ratio was less than 1 in all groups, in the other months it was more than 1, but there was a tendency towards reduction in all groups from May to June. There tended to be an inverse correlation between A/G ratios and faecal egg counts (EPG).

DISCUSSION

The results showed a marked change in the qualitative and quantitative distribution of the different serum proteins during pregnancy. With increasing growth of the foetus there occurred a gradual reduction of the total proteins independent of feeding and anthelmintic treatment reaching the lowest values at the time of lambing. In this respect group 1 made an exception as total serum proteins suddenly rose in April. However, this elevated level might have been accidental as renewed investigations one year later did not show any difference from the other groups. The albumin values were very stable, whereas the globulins, and especially the γ -globulins, were decreasing. This indicates a reduction in the immunoglobulins resulting in a greater risk of infection. The rise in total proteins together with a rise in the globulin fractions in May coincided with parturition. The increase of the globulin fraction (γ -globulins) was especially pronounced in those groups receiving no anthelmintics, and occurred about 3 weeks after the sheep had been moved out on pasture. At that time there was a distinct difference as to the EPG levels of these groups compared with those of groups 1 and 2. In serum protein studies in lambs *Turner & Wilson* (1962) found the A/G ratio and total protein levels to increase with increasing age in lambs free from parasites, and decrease in lambs more or less heavily parasitized. *Perk & Lobl* (1960) also found an increase in the globulins with increasing age in lambs which they thought to be due to an antigenic stress. In adult sheep *Jungmann* (1960) found a decrease in the albumin and β -globulin fractions and an increase in the α - and γ -globulin fractions. *Endrejat* (1956), cited by *Turner & Wilson*, in serum protein studies in parasitized sheep found a decrease in the albumin- and an increase in the β - and γ -globulin levels. So the rise in globulin values in groups 3 and 4 might be due to a gradual increase in antigenic stress caused by parasites or secondary to parasite infestation.

Apart from December the A/G ratios were more than 1, how-

ever, in June the A/G ratios in groups 3 and 4 were 1, and for groups 1 and 2 there were a marked decrease towards 1. A/G ratios more than 1 were found by *Howe* (1925) and cited by *Dukes* (1947). *Perk & Lobl*, however, found the A/G ratio to be more than 1 only in lambs under 3 months of age, while in grown up sheep they found the A/G ratio to be 0.77 based on serum protein studies in 6 lactating ewes. *Koenig et al.* (1949) also found the A/G ratio in lambs to be more than 1 which is in correspondence with the findings of the writer based on 370 electrophoregrams from 44 lambs from birth up to 2 months of age (unpublished data). *Boguth* (1954) investigated the influence of pregnancy on the serum proteins in 5 goats in periods between 68 days ante partem and 38 days post partem. He found a decrease in total protein and albumin levels and a small increase in the globulin fractions. However, it is necessary to perform further investigations in pregnant ewes to see whether the changes in the serum proteins in this condition is a recurring phenomenon and must be allowed for when estimating pathological conditions.

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SUMMARY

Total serum protein levels were determined and filter paper electrophoresis was run in serum samples from 48 ewes during pregnancy under different feeding conditions and anthelmintic treatment.

There was a gradual reduction in total serum protein levels towards lambing in all groups, and also a decrease in the globulin fractions. This caused a rise in A/G from less than 1 to more than 1. After lambing there was a decrease in A/G towards 1, and the fall was most evident in those animals receiving no anthelmintics.

ZUSAMMENFASSUNG

Das totale Serumproteinniveau und das elektrophoretische Bild bei trächtigen Mutterschafen.

Bei verschiedenen Fütterungsverhältnissen und Anthelmintika-Behandlung wurden in Serumproben von 48 Mutterschafen, die in 4 Gruppen verteilt waren, die totale Proteinmenge während der Trächtigkeit bestimmt. Gleichzeitig wurden dieselben Serumproben mit Hilfe von Papierelektrophorese untersucht.

In allen 4 Gruppen wurden die totale Proteinmenge sowie die Globulin-Fractionen gegen Ende der Trächtigkeit nach und nach reduziert. Dieses hatte eine Steigerung des Albumin/Globulin-Verhältnisses von unter 1 bis etwas über 1 zur Folge. Nach dem Lammen wurde ein Fall im A/G-Verhältnis gegen 1 beobachtet. Dieses war besonders auffallend bei den Tieren, die nicht Anthelmintika bekommen hatten.

SAMMENDRAG

Total serumproteinmengde og papirelektroforesemønster hos drektige søyer.

Under ulike foringsforhold og anthelmintica-behandling ble det i serumprøver fra 48 søyer fordelt på 4 grupper i løpet av drektighetstiden foretatt bestemmelser av total protein mengde. Samtidig ble de samme serumprøver undersøkt ved hjelp av papirelektroforese.

I alle 4 grupper forekom en gradvis reduksjon i total proteinmengde og globulin-fraksjoner henimot lammingstiden. Dette resulterte i en økning i A/G fra under 1 opp til over 1. Etter lamming var det en tendens til fall i A/G henimot 1. Dette var mest påfallende hos de dyr som ikke hadde fått anthelmintica.

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