Original Article

TJVR 2019; 3(2): 67-71

Turkish Journal of Veterinary Research

http://www.dergipark.gov.tr/tjvr

e-ISSN: 2602-3695

Profiles of Serum Protein Fractions Pre-Treatment and Post-Treatment in Lambs with Pica Disorder

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Received: 07.05.2019 Accepted: 05.08.2019

ABSTRACT

Objective: The present study was planned to examine the changes in serum protein fractions of lambs with pica symptoms with electrophoresis.

Material and Methods: The three study groups were the control group that included healthy lambs, pre-treatment and post-treatment groups that included lamb with pica. Lambs with pica symptoms were then treated with a single capsule mineral complex orally and single dose vitamin complex. Serum protein fractions in blood samples were determined with cellulose-acetate electrophoresis.

Results: It was determined that in terms of concentration, total protein, albumin and β-globulin, levels did not change, albumin percentage levels increased with pica, however reached control group levels with treatment and α1-Globulin and α2-Globulin levels reduced after treatment. Based on % gr, it was determined that α1-Globulin and β-Globulin did not change and α2-Globulin decreased. ɣ-Globulin level and percentage increased after treatment.

Conclusion: Profiles of serum protein fractions may play an important role in the etiology of pica; it was suggested that the identification of serum protein fractions should be considered as a beneficial method in veterinary clinic for the detection, treatment and follow-up of certain diseases and certain nutrition-related conditions.

Keywords: *Pica disease, Vitamins, Minerals, Serum protein fractions, Serum electrophoresis*

INTRODUCTION

Pica was first used as a term for a perverted craving for substances unfit to be used as food by Ambrose Paré (1509-1590). *Pica* is the medieval Latin name for the bird called the magpie, who, it is claimed, has a penchant for eating almost anything (Şahin *at al*., 2001). Pica is an abnormal and uncontrollable appetite. It is characterized by animals that chew or eat fences, trees, dirt, bones, or other inanimate objects that are not generally accepted as feed. Although the mechanism behind pica was not fully revealed, it was associated with parasites and phosphorus, salt, protein, cobalt, etc. deficiency. It might be related to a number of factors including the imbalance of certain proteins, α-amino acids, vitamins, trace elements, impairment of diet calcium-phosphorus ratio (Aytekin and Kalınbacak, 2008; Smith, 2015; Elshahawy and Aly, 2016). Pica is one of the most significant domestic animal diseases due to potential complications. The condition is seen among dairy cattle, cows (especially pregnant and lactating), other animals

such as sheep and goats (Aytekin at al., 2011; Elshahawy and Aly, 2016).

Serum proteins reflect hundreds of different protein groups present in the blood. Serum proteins are grouped under 5-6 electrophoretic bands for use in typical clinical laboratories. Clinically significant serum proteins possess various functions including circulation of lipids, hormones, vitamins and metalloenzymes in the blood, and regulation of non-cellular activities and the immune system (Metzler and Metzler, 2001; Adkins et al., 2002; Jacobse at al., 2005). Hundreds of proteins dissolve in the plasma. By measuring the concentration of these proteins, the clinician could achieve information on the status of diseases in different organ systems (Akgul et al., 2000).

The present study was planned to determine the changes in serum protein fractions in lambs with pica symptoms and to investigate the effects of vitamin-mineral combination treatment.

MATERIALS and METHODS

The patient group included ten 4-6 months old Akkarman stock lambs with pica symptoms in the present study. The control group included 10 healthy lambs with typical body temperature, pulse and respiration. Blood samples were obtained from the *V. jugularis* of all lambs into ten serum tubes. Lambs with pica symptoms were then treated with a single capsule BAKOSEL orally (Ceva, France: vitamin E 500 IU, dicalcium phosphate 150 mg, sodium selenite 2.5 mg, copper sulfate 10 mg, cobalt sulfate 12.5 mg). Also, single dose POLYFIL (Atafen, Turkey: 0.08 g fosforilkola s, 5,000 I.U. vit A, 0.05 mg of vitamin B12, 0.02 g vit E) was administered. Blood samples were taken from the *V. jugularis* of the animals into serum tubes in the same manner on the 15th day after treatment. Thus, two groups were formed: control and treatment included pre-treatment and post-treatment

Blood samples were collected from all groups, and the serum samples was separated by centrifugation at 500 ×g for 10 min. Total protein concentrations were analyzed using the biuret method. The serum protein fractions were separated using the Helena Lab-Titan III® Serum Protein Electrophoresis device (Cat No. 3023), Helena Lab-Titan III Cellulose acetate cards, and Electra HR Buffer (Cat No. 5805) tampon solutions (Helena, Bioscience Europe, UK) and then stained with the Ponceau S Stain solution. The bands obtained after electrophoresis were evaluated for serum protein fractions in Platinum 3.0 program, and the protein concentrations were determined.

The data were analyzed with a one-way analysis of variance (ANOVA). Duncan's test was applied for multiple comparisons. Differences were considered significant when the p value was less than 0.05 using SPSS 22.0 statistical software.

RESULTS

Clinical Findings

Symptoms that were observed pre-treatment in the lambs with pica disorder: soil eating, wool eating, weight loss, growth deceleration, surface and iron licking, and a wish to eat non-food objects. Pica indications eat non-food objects known to be caused by mineral element deficiency mostly disappeared in with pica disorder post-treatment.

Biochemical Findings

Serum protein concentrations for the lambs with pica are summarized in Table1. Comparison of all study group findings demonstrated that there were no statistically significant changes in total serum protein, albumin and β-globulin concentrations. The α1-Globulin and α2-Globulin concentrations did not change statistically when compared to the control group, however it was determined that both α1-Globulin and α2-Globinin concentrations statistically significantly decreased after the treatment when compared to the control group ($p < 0.05$). The ɣ-Globulin concentration was the lowest in the pre-treatment group ($p <0.05$) and reached the highest values ($p <0.05$) after the treatment when compared to both control and pre-treatment groups.

There was no change in total serum proteins in all study groups. However, proportional change was also considered to show the changes of serum protein fractions in total protein. Table 2 summarizes the data obtained after the analysis of the serum protein fraction % rates in total serum proteins.

Analysis of the albumin values based on % gr demonstrated that there was a statistically significant increase in pretreatment group when compared to the control group ($p <0.05$). The percentage changes in α1-globulin total serum protein demonstrated that the control group had the highest levels statistically, and no significant increase was recorded with treatment. Similarly, it was observed that the α2-Globulin rate decreased to levels that were even lower than the control with

treatment (p <0.05). There was no statistically significant difference between β-globulin levels, although there were relative differences between the study groups. It was calculated that ɣ-Globulin levels were significantly lower level in pre-treatment group when compared to the control, and increased to levels higher than control with treatment (p <0.05). Furthermore, it was determined that the A / G ratio was higher (p <0.05) in the pica group when compared to all other groups.

Tablo 1. Concentrations of serum protein fractions determined by the acetate electrophoresis method in study groups

Serum proteins (g/dl)	Control (n=10)	Treatment (n=10)	
		Pre-treatment	Post-treatment
Total protein	9.829±2.26	9.771±1.83	10.097±0.76
Albumin	4.223±1.27	4.767±0.88	4.675±0.66
α_1-Globulin	1.284±0.42a	0.872±.37ab	0.793±.15b
α_2-Globulin	1.865±0.59a	2.180±0.57a	1.350±0.18b
β-Globulin	1.010±0.36	0.854±.20	0.953±.19
ɣ-Globulin	1.578±0.64b	1.085±0.38a	2.321±0.62c

There is a difference of p < 0.05 between different letters

Tablo 2. Percentage (%) of serum protein fractions determined by the acetate electrophoresis method in study groups

Serum proteins (%)	Control (n=10)	Treatment (n=10)	
		Pre-treatment	Post-treatment
Albumin	42.653±6.12a	49.017±5.16b	46.415±6.39ab
α_1-Globulin	13.39±4.33a	8.831±2.77b	7.857±1.23b
α_2-Globulin	19.173±4.48a	22.152±3.11b	13.396±1.82c
β-Globulin	10.204±2.62	8.745±1.32	9.497±2.00
ɣ-Globulin	16.043±5.55b	11.080±3.24a	22.837±5.20c
A/G	0.765±.20a	0.981±0.20c	0.888±0.22b

There is a difference of p < 0.05 between different letters

DISCUSSION

Serum proteins are important biochemical parameters used for identification, analysis, diagnosis and pathogenesis of diseases. Different methods are used to determine serum protein fractions. One of the most common methods is serum protein electrophoresis. Cellulose acetate electrophoresis is one of electrophoresis methods. This method has been used clinically in human medicine since 1950s (Walker et al., 1990). However, recent studies in veterinary medicine reported a significant increase in the use of cellulose acetate electrophoresis, albeit not as much as in human medicine. Our studies and those conducted by others demonstrated that utilization of the cellulose acetate electrophoresis method in veterinary clinical studies would contribute to the acquisition of healthier, less expensive and rapid results (Wıjnen and Van Dieijen-Visser, 1996; Akdoğan et al., 1999; Apaydın and Dede, 2010; Yüksek et al., 2013; Güllü and Dede, 2016).

Serum proteins are easily influenced by physiological, nutritional, sex, environmental, and genetic factors. Although, a decrease is seen in all bands depending on the development of hypoproteinemia in the cases with malnutrition or severe protein loss, the most significant reduction is in albumin. Enteropathy and nephropathy induced protein loss, causes a decrease in serum albumin, α1, β, and ɣ -globulin levels. Despite the decrease in plasma albumin in chronic hepatocellular disorders, there is no or a very small decrease in plasma albumin in acute liver diseases (Erdal, 1987; Karagül, et al., 2000; Nikvand et al., 2018).

Pica treatment in both humans and animals varies depending on the type and cause of the pica.

Conventional medical treatment may be appropriate in certain situations. For example, supplementation with iron-containing vitamins has been shown to subside craving in some iron-deficient patients (Firyal, 2007).

Several studies were conducted on total serum protein, vitamin, various mineral etc. content in hematological and serum proteins in order to determine the etiology of animals with pica (Akgul et al., 2000; Aytekin and Kalınbacak, 2008; Aytekin et al., 2011). Although several studies were conducted with cellulose acetate electrophoresis to research animal diseases, there was no study on the determination of serum protein fractions with cellulose acetate electrophoresis in both human and veterinary medicine related pica disease, which is eating and swallowing non-nutrient substances such as wires, stones, feathers, hair, etc.

Singh et al. (1986) reported that the total serum protein amount of the camels with pica was lower when compared to the camel without pica in a study conducted with camels. In spectrophotometric measurements conducted to research serum mineral and protein concentrations in horses with and without pica, Aytekin et al. (2011) demonstrated that the total serum protein concentration in horses with pica did not exhibit any difference. In a colorimetric study conducted on serum proteins of cattle with pica and healthy cattle, Nikvand et al. (2018) determined that the total serum protein concentration did not differ in cattle diagnosed with pica. Şahin et al. (2011) reported that serum protein concentrations were significantly below normal levels in lambs with pica.

However, in the present study, contrary to the above-mentioned data, it was determined that the total serum protein concentration in the lambs with pica was close to the control group levels and the post-treatment increase was not significant. It is suggested that this was due to the impact of animal species, nutritional, environmental, etc. factors.

In the conducted literature review, no studies that determined serum albumin concentrations in animals with pica using electrophoresis were found. However, Nikvand et al. (2018) reported that serum albumin concentration did not vary in the colorimetric study they conducted to determine serum protein concentration in cattle with pica.

In the present study conducted with cellulosic acetate electrophoresis method in lambs with pica, it was determined that albumin concentration did not change when compared to the control group, and analysis of the percentage in total serum protein demonstrated that there was no change in albumin concentration and ratio after the treatment with mineral and vitamin supplements.

The comparison of the serum globulin fractions of the study groups demonstrated that the α1-Globulin (α_1-antitrypsin, α1-antichymotrypsin, acid glycoprotein, α1 -lipoprotein) and α2-Globulin (ceruloplasmin, α2-macroglobulin, haptoglobin) concentrations and percentages in total serum protein in the patient group were similar to the control group, and both globulin fractions exhibited a significant decrease ($p<0.05$) after the treatment when compared to the control group. It was concluded that the proteins in these fractions were influenced by pica, which is a nutritional and metabolism disorder. After treatment, decreases compared to control are thought to result from increased metabolic activities in which these proteins are involved and consumption of these proteins in these pathways.

It was observed that group β-globulin concentrations and percentages were not affected by pica.

In the patient group, it was found that ɣ-Globulin concentration and percentage were significantly lower, and after treatment, they increased approximately two-fold and reached a significantly higher concentration when compared to the control group.

It was determined that the A / G ratio in the patient group was higher and approached the control group levels after the treatment.

It was determined that concentrations of various minerals were low in studies conducted with camel, cattle and calf with pica and mineral complex and vitamin supplement treatments led to increased copper and phosphorus levels and the habit of eating non-nutritional foreign objects decreased (Faye and Bengoum, 1994; Aytekin and Kalınbacak, 2008).

In the present study, it was determined that serum total protein and albumin content in animals with pica were not different when compared to the control group, however especially α1-, α2- and ɣ-Globulin fractions and A / G ratio was affected in animals with pica and as a result of the regular use of mineral and vitamin complexes in the treatment of pica, these values approached control group levels. In particular, the improvement in the gamma globulin fraction was considered as an indication of

the strengthening of the immune system. Considering the fact that the proteins in the α1 and α2-globulin fractions were affected by pica, it was concluded that further studies that would research the status of these proteins in pica should be conducted.

In conclusion, it was suggested that the identification of serum protein fractions should be considered as a beneficial method in veterinary clinic for the detection, treatment and follow-up of certain diseases and certain nutrition-related conditions.

ACKNOWLEDGMENTS

Conflict of Interests: The authors declared that there is no conflict of interests.

Financial Disclosure: The authors declared that this study has received no financial support.

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