SHORT COMMUNICATION

SERUM PRODUCTION OF ANDROCTONUS CRASSICAUDA (SCORPION) VENOM: EVALUATING THE IMPACT OF HIGH-QUALITY PROTEIN DIETS

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ABSTRACT

In this study, serum horses were utilized to evaluate the effect of feeding high-quality, protein-enriched diets on antibody production. The horses were divided into two groups, each containing three animals (two females and one male per group). Two feeding periods were implemented, with one group serving as the control and fed a diet containing 13.65% crude protein, while the other group received a trial diet containing 18.05% crude protein. To increase accuracy, the control and experimental groups were alternated in a crossover design.

No statistically significant differences were observed between the trial and control groups ($p \geq 0.05$). Similarly, no significant differences were found when the data from the trial and control groups were analyzed separately by sex. However, a statistically significant difference was found between sexes ($p=0.004$), with antibody titers increasing by 40.2% in the trial groups overall and by 42.6% in male horses.

No statistically significant changes were found for serum total protein values, albumin, and globulin levels ($p>0.05$).

It was observed that individual antibody levels varied considerably, which limited the ability to achieve statistical significance. However, it can be inferred that the quantity and quality of dietary proteins significantly enhance antibody production in quantitative terms.

Keywords: Antibody titer, horse, lysine, methionine

INTRODUCTION

Scorpion stings are frequently encountered in tropical and subtropical climates. This poses a significant public health concern, with some patients experiencing severe clinical manifestations and life-threatening complications (Abroug et al., 2020). Scorpion envenomation may present clinically with a wide array of symptoms, from localized dermal reactions to potentially fatal neurologic, respiratory, or cardiovascular collapse (Reckziegel et.al, 2014). Globally, among the 1.753 known scorpion species, approximately 50 are venomous, with 20-25 considered potentially fatal (Khatony et al., 2015). The annual global incidence of scorpion stings is estimated at 1.200.000 cases, with an associated mortality rate of approximately 3,000 (Isbister, 2014).

In Turkey, *Androctonus crassicauda* is the most common and highly venomous scorpion species, known for its potentially lethal stings. These scorpions are predominantly found in the Southeastern Anatolia Region and certain provinces in the Eastern Anatolia Region (Özkan et al., 2003).

The use of antivenom is currently regarded as the most effective treatment for envenomed patients. Consequently, further research is necessary to develop more specific and purified antivenoms to improve the treatment of scorpion sting victims. According to Russell et al. (1964), antivenom remains the sole definitive treatment for such cases.

In Turkey, the venom of *Androctonus crassicauda* is the preferred antigen for antivenom production. This venom induces immunity more effectively in animals, and fatalities among animals used in antivenom production are rare. Additionally, the broad paraspecific efficacy of antivenom prepared against *A. crassicauda* suggests its potential utility in treating stings from various scorpion species both within Turkey and globally (Filazi et al., 2020).

In the treatment of scorpionism cases, antivenoms are produced by injecting venom into animals such as sheep, goats, and horses, followed by the periodic collection of antibodies. Horses are often used as hyperimmunized animals in the antivenom production process due to their ability to produce large volumes of antiserum, providing a significant advantage (Theakston et al., 2003). However, their use may result in higher costs compared to other animals (Pratanaphon et al., 1997).

Horses belong to the group of monogastric herbivores adapted to a specialized diet.It is recommended to add high-quality protein to meet the daily crude protein requirements of horses. Methionine, lysine, and threonine can be included in the diet as sources of high-quality protein. These added proteins are primarily used to increase metabolic energy, particularly in horses with a negative energy balance (Laurie 2008; NRC 2007).

However, studies investigating the relationship between nutrition and immune function in horses are limited (Maggini et al., 2007). The dynamic interaction between nutrient availability and nutritional status significantly influences immune health and disease resistance (Scrimshaw et al., 1968; Calder and Jackson, 2000). Recent studies highlight that amino acids play significant roles in the development of cytotoxic immune cells such as T cells, B cells, and macrophages; in facilitating cellular chemical reactions, lymphocyte proliferation, and gene expression; and in promoting cytokine production, antibody synthesis, and the regulation of immune responses (Peng et al., 2007)

The aim of this study is to assess the effects of high-quality proteins feeding on the immune system of horses that have been acquired with scorpion serum.

MATERIAL AND METHODS

Approval of the Ethics Committee

This study was approved by Ankara Universıty Animal Experiment Ethical Commitee (date and permit no: 18/03/2020, 2020-6-48).

Animals and diet

The study sample consisted of six native horses aged 4 to 8 years, with a body weight ranging from 350 to 400 kg. This experiment was conducted with two groups of horses, one experimental and one control, each consisting of one male and two females (n=3). Horses were randomly assigned into two groups.

In our study, we investigated the efficacy of scorpion venom serum under two different dietary conditions. Due to the small sample size (N=3 horses/treatment), a repeated study design was implemented to increase statistical power and account for individual variability. Initially, the horses were divided into two groups: control and experimental, with three horses in each group.

To ensure robust results, after the first phase of the experiment, the group assignments were switched. Horses initially in the control group were moved to the experimental group, and vice versa. This crossover design allowed all horses to serve as their own controls at different stages of the study, effectively doubling the number of observations (N=6 horses/treatment) and reducing variability caused by individual differences in response to treatments.

The horses us ed for the production of scorpion serum were housed individually in compartments and fed with either control or experimental diets for a period of four months. The control and experimental groups were alternated to account for potential individual differences and ensure the reliability of the findings.

The concentrate feed used in the experiment was prepared at the Faculty of Veterinary Medicine Education, Research and Application Center (Ankara University), utilizing supplied raw materials. Barley straw and dried alfalfa grass were provided as roughage for both groups. Additionally, the feed for the experimental group was supplemented with lysine-rich soybean meal and methionine to enhance protein quality.

Three horses were randomly assigned to each of a control and an experimental group. To account for individual variability, a repeated study design was implemented, with the grouping rearranged accordingly. Each horse was fed daily with 4 kg of concentrated feed, 3 kg of alfalfa hay, and 2 kg of barley straw. The control group received a diet composed of 80.5% barley straw and alfalfa hay, and 15.7% concentrate mix. In contrast, the experimental group was fed a diet consisting of 66% barley straw and alfalfa hay, and 30.2% concentrate mix.Water was provided *ad libitum* to all horses.

Table 1 Composition and chemical analysis of concentrated diet

Composition		
	Control Group	**Experimental Group**
Barley Straw (g/kg)	400.00	400.00
Alfalfa Grass (g/kg)	405.00	260.00
Corn (g/kg)	100.00	100.00
DDGS (g/kg)	57.00	57.00
Soybean Meal,CP 48 % (g/kg)	0	143.00
Limestone (g/kg)	3000	30.00
Salt (g/kg)	7.00	7.00
Vitamin-mineral Premix*(g/kg)	1.00	1.00
DL-Methionine **(g/kg)	0.00	2.00
Total	1000	1000

Composition		
	Control Group	**Experimental Group**
Chemical Composition		
Dry matter (%)	90.94	91.60
Crude protein (%)	13.65	18.05
Crude fiber (%)	6.26	5.71
Crude oil (%)	2.97	2.60
Crude ash (%)	6.18	6.35
Calcium (%)	1.10	1.37
Available Phosphorus (%)	0.45	0.46

***Vitamin-mineral Premix:** Vitamin D3, Vitamin A, Vitamin E, Selenium, Manganese, Cobalt, Iodine, Iron, Zinc, Copper, Calcium Carbonat. ****DL-Methionine origin:** France.

Experiment design

Scorpion antiserum was produced in horses housed at the Ankara University Faculty of Veterinary Education Research and Practice; an institution affiliated with the General Directorate of Public Health under the Ministry of Health. To enhance antibody production for scorpion antiserum, high-quality, protein-rich diets were prepared for the horses. One week prior to venom administration and throughout the entire study, the experimental group was fed a protein-enriched diet.The antigenized scorpion venom was administered intramuscularly with adjuvants. During the first feeding period, horses received their initial venom injections, which were repeated seven times at one-week intervals. In the second feeding period, venom injections were administered for one week,with a total of four applications.

This approach ensured that the small sample size did not compromise the validity of the results, as each animal's response under both dietary conditions was evaluated.

Blood sampling

Antibody titers were assessed 1–3 days after the final injection. Blood samples were collected via venipuncture of the external jugular vein from each horse, using 10 ml vacuum tubes without anticoagulant. After centrifugation, serum samples were stored in tubes at +4°C for 24 hours prior to conducting laboratory analyses. A complete blood count was conducted to measure the size of red blood cells, hemoglobin levels, white blood cell counts, platelet counts, and blood protein concentrations, including albumin and globulin, utilizing a photometric method for analysis..

A two-phase feeding program was implemented in this study, with the second phase involving the reassignment of horses between control and experimental groups to enhance the accuracy of the findings. We determined higher concentrations of total protein and globulin, while albumin level decreased. However, these changes were not statistically significant ($p>0.05$).

Scorpion neutralizing antibody titer

For each venom type provided by manufacturers, the LD_{50} of the scorpion venom contained within the antiserum requires verification.The manufacturer's LD_{50} value serves as the median dilution for this purpose; four additional dilutions, two lower and two higher, are prepared using a dilution factor of 1,2. Each of these dilutions is intravenously injected at a volume of 0.25 ml into four mice weighing 18-20 grams. The same gender of mice used in the LD_{50} determination must also be used in the potency test. After a 48-hour observation period, the data obtained is analyzed using the Sperman-Kärber method.

The venom of *Androctonus crassicauda* scorpions, collected by means of electrical stimulation, is utilized as an antigen following the determination of its LD_{50} in mice weighing 18–20 grams. For each horse, the antigen and adjuvant (aluminum potassium sulfate extra pure crystalline) were prepared in a fixed volume of five milliliters for injection.

Table 2 Steps for Using Venom

First-period feeding		Second-period feeding	
Time (week)	Venom dose (LD_{50})	Time (week)	Venom dose (LD_{50})
1	5	1	50
2	10	2	100
3	25	3	250
4	50	4	400
5	100	5	-
6	250	6	-
7	400	7	-

Statistical analysis

The data were analyzed using the Mann-Whitney U test, with statistical significance set at $p<0.05$.

RESULTS

Each horse was injected with venom subcutaneously to measure blood serum parameters and antibody titer.

Table 3 Blood serum parameters and antibody titer across different experimental groups

Parameters	**n**	**Control Group** $\bar{x}\pm S\bar{x}$	**Experimental Group** $\bar{x}\pm S\bar{x}$	**p-value**
Antibody Titer (NU/ml)*	6	46.87± 40.31	61.14 ± 65.76	0.660
Total protein (g/dl)**	6	8.51± 0.62	8.63± 0.73	0.767
Albumin (g/dl)	6	5.23 ± 0.44	5.13± 0.33	0.690
Globulin (g/dl)	6	3.28± 0.32	3.49 ± 0.54	0.428

Difference between groups is statistically insignificant (p>0,05).

*NU/ml: Antiserum potency per milliliter that neutralizes the LD_{50} of *A. Crassicauda* venom.**g/dl: grams per deciliter.

Table 4 Blood serum parameters and antibody titers in horses of different genders during the experiment

Female				
Parameters	**n**	**Control Group** $\bar{x}\pm S\bar{x}$	**Experimental Group** $\bar{x}\pm S\bar{x}$	**p-value**
Antibody Titer (NU/ml)*	4	22.63 ± 16.01	23.72 ± 15.22	0.773
Total protein (g/dl)**	4	8.54± 0.59	8.68± 0.92	0.988
Albumin (g/dl)	4	5.33 ± 0.51	5.19± 0.42	0.564
Globulin (g/dl)	4	3.21± 0.09	3.49 ± 0.34	0.555
Male				
Antibody Titer (NU/ml)	2	8.44± 0.94	8.53± 0.28	0.980
Total protein (g/dl)	2	5.03 ± 0.26	5.03± 0.98	0.891
Albumin (g/dl)	2	3.41± 0.67	3.50 ± 0.18	0.986
Globulin (g/dl)	2	95.37 ± 17.39	136.00 ± 64.20	0.439

Difference between groups is statistically insignificant (p>0,05).

*NU/ml: Antiserum potency per milliliter that neutralizes the $LD_{50}$of *A. Crassicauda* venom.**g/dl: grams per deciliter.

Table 5 Blood serum parameters and antibody titers in male and female horses involved in the experiment

Parameters	Gender		p-value
	Male(n=2) $\bar{x}\pm S\bar{x}$	Female(n=4) $\bar{x}\pm S\bar{x}$	
Antibody Titer (NU/ml)*	115.68^{a}**± 45**.00	23.178^{b}**± 14**.47	0.004
Total protein(g/dl)**	8.48 ± 0.56	8.6 ± 0.72	0.979
Albumin(g/dl)	5.08 ± 0.21	5.26 ± 0.44	0.885
Globulin (g/dl)	3.40 ± 0.39	3.35 ± 0.47	0.806

The difference between the antibody titers indicated by different letters (a, b) is statistically significant (p<0.05). Difference between groups is statistically insignificant (p>0,05).

*NU/ml: Antiserum potency per milliliter that neutralizes the LD_{50} of *A. Crassicauda* venom.**g/dl: grams per deciliter.

Hematological analysis

At the end of the two study periods, blood samples were collected from the jugular vein of horses into purple-capped tubes containing EDTA. Hematological analyses were conducted at the Central Research Center of the Faculty of Veterinary Medicine, Ankara University.

Table 6 Hematological parameters of control and experimental groups

	n	Control Group	Experimental Group	p-value
WBC (10^9/L)	6	10.76± 2.50	11.06 ±1.68	0.813
LYM (10^9/L)	6	3.03 ± 0.93	2.76 ± 1.05	0.652
MONO (10^9/L)	6	0.78 ± 0.29	0.81 ± 0.23	0.831
NEUT (10^9/L)	6	6.85 ± 2.60	6.15 ± 3.87	0.721
EOS (10^9/L)	6	0.10 ± 0.15	0.01 ± 0.04	0.231
LYM (%)	6	29.26 ± 9.42	26.48 ± 11.84	0.662
MONO (%)	6	6.85 ± 2.33	6.91 ± 2.36	0.962
NEU(%)	6	62.76 ± 12.02	53.70 ± 29.46	0.501
EOS (%)	6	1.11 ± 1.75	0.23 ± 0.52	0.264
RBC (10^{-2}/L)	6	8.35 ± 1.35	7.94 ± 1.39	0.615
HGB (g/dL)	6	15.38 ± 2.08	14.73 ± 2.13	0.605
HCT(%)	6	42.00 ± 5.77	39.45 ± 5.90	0.467
MCV (fL)	6	50.53 ± 3.86	49.91 ± 3.71	0.784
MCH (pg)	6	18.50 ± 1.14	18.70 ± 1.27	0.781
MCHC (g/dL)	6	36.66 ± 0.89	37.48 ± 1.19	0.211
RDWa (fL)	6	27.03 ± 2.01	26.21 ± 2.32	0.530
RDW (%)	6	15.93 ± 0.71	16.03 ± 0.73	0.817
PLT (10^9/L)	6	142.33 ± 26.50	160.83 ± 53.43	0.465
MPV (fL)	6	5.66 ± 0.37	5.68 ± 0.44	0.945

Difference between groups is statistically insignificant (p>0.05). WBC - white blood cell . NEUT - neutrophil. LYM - lymphocyte . MONO - monocyte . NEU – neutrophil. EOS - eosinophi . RBC – red blood cells. HGB –haemoglobin. HCT - haematocrite. MCV – mean cell volume. PLT – platelets. MPV - mean platelet volume . MCH- mean corpuscular hemoglobin. MCHC- mean corpuscular hemoglobin concentration. RDW- red blood cell distribution width. RDWa- red cell distribution width (absolute)

Table 7 Hematological parameters of female horses in control and experimental groups

	N	Control Group $\bar{x}\pm S\bar{x}$	Experimental Group $\bar{x}\pm S\bar{x}$	p-value
WBC (10^9/L)	4	11.07±2.58	10.87± 1.96	0.564
LYM (10^9/L)	4	3.55 ± 0.62	3.35± 0.64	0.564
MONO (10^9/L)	4	0.92 ± 0.25	0.95± 0.12	0.963
NEUT (10^9/L)	4	6.52 ± 2.65	6.55± 2.29	0.773
EOS (10^9/L)	4	0.07 ± 0.15	0.02± 0.05	0.850
LYM (%)	4	33.37 ± 8.12	32.27± 9.44	0.960
MONO (%)	4	8.00 ± 1.71	8.20± 1.49	0.564
NEU(%)	4	57.90 ± 10.33	59.15± 10.32	0.564
EOS (%)	4	0.72 ± 1.45	0.35± 0.63	0.741
RBC (10^{-2}/L)	4	9.02 ± 1.00	8.55± 1.31	0.882
HGB (g/dL)	4	16.42 ± 1.26	15.85± 1.60	0.772
HCT(%)	4	45.05± 3.38	42.82± 3.46	0.386

	N	Control Group x̄±Sx̄	Experimental Group x̄±Sx̄	p-value
MCV (fL)	4	50.22± 4.95	50.55±4.62	0.077
MCH (pg)	4	18.30± 1.40	18.70± 1.60	0.772
MCHC (g/dL)	4	36.50± 0.94	37.00± 0.85	0.248
RDWa (fL)	4	27.27± 2.53	27.25± 2.12	0.773
RDW (%)	4	16.22± 0.65	16.17± 0.79	0.773
PLT (10^9/L)	4	131.75± 26.51	144.00± 50.83	0.564
MPV (fL)	4	5.65± 0.26	5.75± 0.41	0.561

Difference between groups is statistically insignificant (p>0,05). WBC - white blood cell . NEUT - neutrophil. LYM - lymphocyte. MONO - monocyte . NEU – neutrophil. EOS - eosinophil. RBC – red blood cells. HGB –haemoglobin. HCT - haematocrite. MCV – mean cell volume. PLT – platelets. MPV - mean platelet volume. MCH- mean corpuscular hemoglobin. MCHC- mean corpuscular hemoglobin concentration. RDW- red blood cell distribution width. RDWa - red cell distribution width (absolute)

Table 8 Hematological parameters of male horses in control and experimental groups

	N	Control Group x̄±Sx̄	Experimental Group x̄±Sx̄	p- value
WBC (10^9/L)	2	10.15 ±3.18	11.45 ± 1.48	0.439
LYM (10^9/L)	2	2.00 ± 0.00	1.60 ± 0.42	0.102
MONO (10^9/L)	2	0.50 ± 0.00	0.55 ± 0.07	0.317
NEUT (10^9/L)	2	7.50 ± 3.39	5.35 ± 7.56	0.948
EOS (10^9/L)	2	0.15 ± 0.21	0.00 ± 0.00	0.317
LYM (%)	2	21.05 ± 6.57	14.90 ± 5.65	0.439
MONO (%)	2	4.55 ± 1.62	4.35 ± 1.20	0.992
NEU(%)	2	72.50 ± 10.88	42.80 ± 60.52	0.969
EOS (%)	2	1.90 ± 2.68	0.00 ± 0.00.	0.317
RBC (10^{12}/L)	2	7.02 ± 0.91	6.71 ±0.20	0.987
HGB (g/dL)	2	13.30 ± 1.97	12.50 ± 0.00	0.889
HCT(%)	2	35.90 ± 4.52	32.70 ± 1.27	0.439
MCV (fL)	2	51.15 ± 0.21	48.65 ± 0.49	0.121
MCH (pg)	2	18.90 ± 0.42	18.70 ± 0.56	0.439
MCHC (g/dL)	2	37.00 ± 0.98	38.45 ± 1.48	0.439
RDWa (fL)	2	26.55 ± 0.49	24.15 ± 0.77	0.121
RDW (%)	2	15.35 ± 0.49	15.75 ± 0.77	0.439
PLT (10^9/L)	2	163.50 ± 7.77	194.50 ± 55.86	0.896
MPV (fL)	2	5.70 ± 0.70	5.55 ± 0.63	0.439

Difference between groups is statistically insignificant (p>0,05). WBC - white blood cell . NEUT - neutrophil. LYM - lymphocyte. MONO - monocyte. NEU – neutrophil. EOS - eosinophil. RBC – red blood cells. HGB –haemoglobin. HCT - haematocrite. MCV – mean cell volume. PLT – platelets. MPV - mean platelet volume. MCH- mean corpuscular hemoglobin. MCHC- mean corpuscular hemoglobin concentration. RDW- red blood cell distribution width. RDWa- red cell distribution width (absolute)

Table 9 Hematological parameters of male and female horses in control and experimental groups

	Female (n=4) $\bar{x}\pm S\bar{x}$	Male (n=2) $\bar{x}\pm S\bar{x}$	p-value
WBC (10^9/L)	10.97 ±2.12	10.80 ± 2.16	0.80
LYM (10^9/L)	**3.45^a ± 0.59**	**1.80^b ± 0.33**	**0.004**
MONO (10^9/L)	**0.93^a ± 0.18**	**0.52^b ± 0.05**	**0.004**
NEUT (10^9/L)	6.53 ± 2.29	6.42 ± 4.94	0.93
EOS (10^9/L)	0.05 ± 0.10	0.07 ± 0.15	0.93
LYM (%)	32.82 ± 8.17	17.97 ± 6.13	0.01
MONO (%)	**8.10^a ± 1.49**	**4.45^b ± 1.17**	**0.004**
NEU(%)	58.52 ± 9.58	76.62 ± 8.82	0.01
EOS (%)	0.53 ± 1.05	0.95 ± 1.90	0.93
RBC (10^{-2}/L)	**8.78^a± 1.11**	**6.86^b ±0.57**	**0.008**
HGB (g/dL)	**16.13^a± 1.37**	**12.90^b ± 1.23**	**0.008**
HCT(%)	**43.93^a ± 3.38**	**34.30^b ± 3.28**	**0.008**
MCV (fL)	50.38 ± 4.43	49.90 ± 1.47	0.57
MCH (pg)	18.50 ± 1.41	18.80 ± 0.42	0.80
MCHC (g/dL)	36.75 ± 0.87	37.72 ± 1.32	0.21
RDWa (fL)	27.26 ± 2.16	25.35 ± 1.48	0.10
RDW (%)	16.20 ± 0.67	15.55± 0.58	0.15
PLT (10^9/L)	137.87 ± 38.103	179 ± 37.15	0.21
MPV (fL)	5.70 ± 0,32	5.62 ± 0.55	0.80

Difference between groups is statistically insignificant (p>0,05). WBC- white blood cell . NEUT- neutrophil. LYM- lymphocyte . MONO- monocyte. NEU – neutrophil. EOS- eosinophil. RBC – red blood cells. HGB –haemoglobin. HCT- haematocrite. MCV – mean cell volume. PLT – platelets. MPV- mean platelet volume. MCH- mean corpuscular hemoglobin. MCHC- mean corpuscular hemoglobin concentration. RDW- red blood cell distribution width. RDWa - red cell distribution width (absolute)

DISCUSSION AND CONCLUSION

In this study, variations in gamma-globulin, total protein, globulin, and other blood parameters were observed in the horses. However, no significant differences were found in antibody titer, total protein, albumin, and globulin levels between horses fed high-quality protein diets and those fed standard diets. Although statistical significance was not achieved, the 30.45% increase in antibody titer observed in the experimental group is noteworthy. Furthermore, male horses exhibited significantly higher antibody titers (p=0.004) compared to females.

Previous studies have assessed the impact of high-quality protein diets on muscle density and energy metabolism in horses. Waghmarea et al. (2014) investigated the immune response of 33 horses randomly assigned to four groups, receiving subcutaneous injections of cobra venom at two-week intervals. Over the immunization period, biochemical and hematological parameters were monitored at baseline and on weeks 14, 21, 30, and 42. Although mean hemoglobin values initially declined, they later normalized. Serum total protein and globulin levels increased across all groups, while albumin levels showed a slight decrease. No significant changes were noted in

serum creatinine, bilirubin, alkaline phosphatase, or blood urea nitrogen levels. Similarly, in our study, while total protein, albumin, and globulin concentrations differed between groups after exposure to scorpion venom, these differences were not statistically significant.

In a study on rats, diets supplemented with wheat gluten, soy protein, lysine, and methionine increased hepatic nitrogen levels and weight gain. However, supplementation with only soy protein and methionine led to a reduction in antibody titer, whereas lysine and wheat gluten supplementation had no effect (Smith and Kenney, 1969). Consistently, our experimental group, provided with a lysine- and methionine-enriched diet, exhibited no significant difference in antibody titers compared to controls.

In a study conducted by Pablack et al. (2017), three dietary regimens varying in protein quality (low, medium, and high) were administered to 10 adult cats over six weeks in a randomized crossover design. Blood analyses revealed that lower protein quality was associated with increased phagocytic activity of blood monocytes and reduced eosinophil counts. However, our study found no significant differences in WBC, lymphocyte, or monocyte values between groups. Similarly, Sahlu et al. (1992) demonstrated that plasma total protein levels significantly increased with higher protein diets in female Angora goats. In contrast, our study did not show a significant increase in total protein levels in horses fed diets with varying protein content.

This study evaluated the effects of dietary protein and amino acid levels on antibody titer, serum total protein, albumin, globulin, and hematological parameters in horses used for scorpion serum production. The results confirmed that serum albumin and total protein levels remained within physiological limits. However, individual variability in serum production was substantial. This suggests that identifying and utilizing horses with high serum yields could enable more efficient production, reducing the number of animals required.

The large individual variation among horses limited the statistical significance of the positive effects of protein quantity and quality on antibody production. Future studies should focus on well-defined, homogeneous groups with sufficient sample sizes to better evaluate changes in antibody titers and their additive effects.

One of the key findings of this study is the considerable variability in antibody production among horses. It was concluded that production costs could be significantly reduced by selecting horses on proper diets with high antivenom production capacity.

The results of our research are somewhat limited due to the small sample size used in the experiment. Further studies involving a larger number of horses are needed to identify any potential differences between the experimental and control groups.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTION

Conception: TA, MAK, AŞ; Design: TA, AÇ; Supervision: TA, MAK, AŞ; Materials: TA, MAK, AŞ, AÇ; Data Collection: TA; Analysis of Data: TA, AÇ; Literature Review: TA, AÇ, AŞ; Writing: TA; Critical Review: MAK, AÇ, AŞ

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PROIZVODNJA SERUMA IZ VENOMA *ANDROCTONUS CRASSICAUDE* (ŠKORPION): PROCJENA UTJECAJA VISOKOKVALITETNE PROTEINSKE PREHRANE

SAŽETAK

U ovom istraživanju smo na konjskom serumu procjenjivali učinak visokokvalitetne, proteinski obogaćene prehrane na proizvodnju antitijela. Konji su podijeljeni u dvije grupe sa po tri životinje (dvije ženke i jedan mužjak). Životinje su prehranjivane dvaput, pri čemu je jedna grupa korištena kao kontrolna i prehranjivana je sa 13.65% sirovih proteina, dok je ispitivana grupa prehranjivana sa 18,05% sirovih proteina. Da bi se povećala preciznost, kontrolna i ispitivana grupa su se smjenjivale u ukriženom dizajnu studije.

Između ispitivane i kontrolne grupe nisu uočene statistički signifikantne razlike ($p \geq 0.05$). Također, nisu uočene signifikantne razlike kada su podaci iz ispitivane i kontrolne grupe odvojeno analizirani po spolu. Međutim, uočena je statistički signifikantna razlika među spolovima ($p=0.004$), pri čemu su titrovi antitijela ukupno porasli za 40.2% u ispitivanoj grupi, a za 42.6% kod mužjaka.

Nisu uočene statistički sigifikantne razlike za vrijednosti ukupnih serumskih proteina, albumina i globulina ($p>0.05$).

Uočeno je da su pojedinačni titrovi antitijela znatno varirali, što je ograničilo mogućnost postizanja statističke signifikantnosti. Međutim, možemo zaključiti da količina i kvalitet proteina u prehrani značajno povećavaju proizvodnju antitijela u kvantitativnom smislu.

Ključne riječi: Konj, lizin, metionin, titar antitijela