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ORIGINAL ARTICLE

The Effect of Immunostim on Sheep's Immune System

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ABSTRACT
The immune system is a system of biological structures and processes within an organism that protects against disease. The aim of present study was to evaluate the effect of immunostim on sheep's immune response. In present study, 12 Ghezel ram in the same age range were selected. Animals were divided into the 2 identical groups (control and treatment). For evaluation of the effect of the drug, blood samples were obtained before the administration and on weeks 1, 2, 3 and 4 after administration. Total serum protein, Albumin, Globulin, Gama Globulin, IgM, IgG and IgA were measured. Data showed that all parameters have significant difference in compared with control group. In conclusion can be state that use of immunostim has protective effect on sheep's immune response.
Keywords: *immunostim, sheep, immune system, IgM, IgG, IgA.*

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INTRODUCTION

The immune system is a system of biological structures and processes within an organism that protects against disease. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue. Pathogens can rapidly evolve and adapt, and thereby avoid detection and neutralization by the immune system, however, multiple defense mechanisms have also evolved to recognize and neutralize pathogens. Even simple unicellular organisms such as bacteria possess a rudimentary immune system, in the form of enzymes that protect against bacteriophage infections. Other basic immune mechanisms evolved in ancient eukaryotes and remain in their modern descendants, such as plants and insects. These mechanisms include phagocytosis, antimicrobial peptides called defensins, and the complement system. Jawed vertebrates, including humans, have even more sophisticated defense mechanisms [1], including the ability to adapt over time to recognize specific pathogens more efficiently. Adaptive (or acquired) immunity creates immunological memory after an initial response to a specific pathogen, leading to an enhanced response to subsequent encounters with that same pathogen. This process of acquired immunity is the basis of vaccination.

Disorders of the immune system can result in autoimmune diseases, inflammatory diseases and cancer [2,3]. Immunodeficiency occurs when the immune system is less active than normal, resulting in recurring and life-threatening infections. In humans, immunodeficiency can either be the result of a genetic disease such as severe combined immunodeficiency, acquired conditions such as HIV/AIDS, or the use of immunosuppressive medication. In contrast, autoimmunity results from a hyperactive immune system attacking normal tissues as if they were foreign organisms. Common autoimmune diseases include Hashimoto's thyroiditis, rheumatoid arthritis, diabetes mellitus type 1, and systemic lupus erythematosus. Immunology covers the study of all aspects of the immune system.

Microorganisms or toxins that successfully enter an organism encounter the cells and mechanisms of the innate immune system. The innate response is usually triggered when microbes are identified by pattern recognition receptors, which recognize components that are conserved among broad groups of microorganisms [4], or when damaged, injured or stressed cells send out alarm signals, many of which (but not all) are recognized by the same receptors as those that recognize pathogens [5]. Innate immune

defenses are non-specific, meaning these systems respond to pathogens in a generic way [6]. This system does not confer long-lasting immunity against a pathogen. The innate immune system is the dominant system of host defense in most organisms [7].

The herbs used in Immunostim are ecologically clean. They are gathered in the most clean Bulgarian areas – the Rodopa, Rila, Pirin and Stara Planina Mountains. It is designed to be taken by the patients with maximum ease. Does not contain preservatives, coloring agents and stabilizers. The inulin is a prebiotic obtained from the plant cicoria and represents diet, soluble fibers with strongly favorable effect on health. Reception of inulin: increases the absorption of microelements – Ca, Mg, Fe and Zn; strengthens the bone-joint system; balances the stomach acidity; increases immunity; helps for the growth of the beneficial bacteria – Lactobacillus and Bifidus. In the large intestine, inulin produces butyric acid, which has anti-cancer effect.

Immunostim has no undesired or side effects. Despite this, it is not recommended to be taken in pregnancy and breast-feeding, because of the physiological changes in the female organism during this period. The product has been offered on the market for 8 years and the tolerance towards it is proven positive. Overdosage with Immunostim is not possible. Immunostim could not harm you because it is a real natural product, beneficial to your health. It is not addictive and no allergic and toxic effects are observed. The aim of present study was to evaluate the effect of immunostim on sheep's immune response.

MATERIALS AND METHODS

In present study, 12 Ghezel ram in the same age range were selected. Animals were divided into the 2 identical groups (control and treatment). Animals were fed with same diet. 15 days prior the study, animals received albendazole with 15 days interval orally. Animals in the control group received placebo and animals in the treatment group received immunostim at the dose of 1026 mg/case (9 tablets per case). For evaluation of the effect of the drug, blood samples were obtained before the administration and on weeks 1, 2, 3 and 4 after administration. Total serum protein, Albumin, Globulin, Gama Globulin, IgM, IgG and IgA were measured. Data were analyzed using SPSS ver. 18. T-test was used to compare between groups. Also, ANOVA was used to compare the variables in different times.

RESULTS AND DISCUSSION

Comparison of data in term of protein in groups:

Data showed that the serum value of proteins in the groups is significant (table 1).

Table 1: data obtained from measurement of protein and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	6.54±0.11	0.28	0.451
	Treatment	6.43±0.08	0.19	
1	Control	6.55±0.13	0.33	0.593
	Treatment	6.58±0.12	0.30	
2	Control	6.84±0.13	0.33	0.873
	Treatment	6.82±0.08	0.20	
3	Control	7.10±0.10	0.25	0.394
	Treatment	7.24±0.11	0.28	
4	Control	7.72±0.14	0.34	0.537
	Treatment	7.81±0.01	0.04	

Comparison of data in term of Albumin in groups:

Data showed that the serum value of Albumin in the groups is significant (table 2).

Table 2: data obtained from measurement of Albumin and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	3.48±0.14	0.36	0.336
	Treatment	3.69±0.14	0.36	
1	Control	3.74±0.06	0.16	0.930
	Treatment	3.72±0.13	0.32	
2	Control	3.72±0.11	0.28	0.439
	Treatment	3.60±0.10	0.24	
3	Control	3.61±0.08	0.20	0.798
	Treatment	3.65±0.11	0.27	
4	Control	3.86±0.11	0.28	0.079
	Treatment	3.50±0.13	0.34	

Comparison of data in term of Globulin in groups:

Data showed that the serum value of Globulin in the groups is significant (table 3).

Table 3: data obtained from measurement of Globulin and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	3.06±0.17	0.43	0.243
	Treatment	2.73±0.19	0.46	
1	Control	2.81±0.11	0.29	0.608
	Treatment	2.93±0.18	0.44	
2	Control	3.12±0.24	0.59	0.729
	Treatment	3.21±0.10	0.26	
3	Control	3.48±0.14	0.34	0.671
	Treatment	3.58±0.18	0.46	
4	Control	3.86±0.14	0.35	0.051
	Treatment	4.31±0.14	0.34	

Comparison of data in term of Gama-Globulin in groups:
Data showed that the serum value of Gama-Globulin in the groups is significant (table 4).

Table 4: data obtained from measurement of Gama-Globulin and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	6.54±0.11	0.28	0.451
	Treatment	6.43±0.08	0.19	
1	Control	6.55±0.13	0.33	0.593
	Treatment	6.58±0.12	0.30	
2	Control	6.84±0.13	0.33	0.873
	Treatment	6.82±0.08	0.20	
3	Control	7.10±0.10	0.25	0.394
	Treatment	7.24±0.11	0.28	
4	Control	7.72±0.14	0.34	0.537
	Treatment	7.81±0.01	0.04	

Comparison of data in term of IgM in groups:
Data showed that the serum value of IgM in the groups is significant (table 5).

Table 5: data obtained from measurement of IgM and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	250.33±14.65	35.89	0.237
	Treatment	223.33±15.70	38.47	
1	Control	230.00±9.67	23.69	0.616
	Treatment	239.16±14.81	36.29	
2	Control	254.66±19.94	48.86	0.716
	Treatment	262.83±8.75	21.45	
3	Control	281.33±10.55	25.85	0.581
	Treatment	292.00±15.40	37.74	
4	Control	313.33±12.40	30.38	0.071
	Treatment	349.83±13.17	32.28	

Comparison of data in term of IgG in groups:
Data showed that the serum value of IgG in the groups is significant (table 6).

Table 6: data obtained from measurement of IgG and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	1085.33±63.30	155.05	0.240
	Treatment	969.16±68.18	167.02	
1	Control	997.50±41.94	102.73	0.611
	Treatment	1078.00±50.01	122.48	
2	Control	1090.33±88.89	217.74	0.622
	Treatment	1139.50±38.18	93.53	
3	Control	1219.66±45.68	111.91	0.577
	Treatment	1266.16±66.51	162.93	
4	Control	1358.33±53.83	131.87	0.073
	Treatment	1515.50±57.05	139.75	

Comparison of data in term of IgA in groups:
Data showed that the serum value of IgA in the groups is significant (table 7).

Table 7: data obtained from measurement of IgA and comparison between groups

Time (week)	Group	Mean±SE	SD	P-value
Before immunostim	Control	334.00±19.45	47.66	0.241
	Treatment	298.33±20.99	51.41	
1	Control	307.00±12.90	31.61	0.611
	Treatment	319.33±19.66	48.16	
2	Control	339.66±26.53	64.99	0.713
	Treatment	350.66±11.77	28.83	
3	Control	375.33±14.05	34.42	0.576
	Treatment	389.66±20.44	50.07	
4	Control	418.00±16.57	40.59	0.072
	Treatment	466.67±17.61	43.14	

Immune response

Inflammation

Inflammation is one of the first responses of the immune system to infection [8]. The symptoms of inflammation are redness, swelling, heat, and pain, which are caused by increased blood flow into tissue. Inflammation is produced by eicosanoids and cytokines, which are released by injured or infected cells. Eicosanoids include prostaglandins that produce fever and the dilation of blood vessels associated with inflammation, and leukotrienes that attract certain white blood cells (leukocytes) [9,10]. Common cytokines include interleukins that are responsible for communication between white blood cells; chemokines that promote chemotaxis; and interferons that have anti-viral effects, such as shutting down protein synthesis in the host cell [11]. Growth factors and cytotoxic factors may also be released. These cytokines and other chemicals recruit immune cells to the site of infection and promote healing of any damaged tissue following the removal of pathogens [12].

Complement system

The complement system is a biochemical cascade that attacks the surfaces of foreign cells. It contains over 20 different proteins and is named for its ability to "complement" the killing of pathogens by antibodies. Complement is the major humoral component of the innate immune response [13, 14]. Many species have complement systems, including non-mammals like plants, fish, and some invertebrates [15].

In humans, this response is activated by complement binding to antibodies that have attached to these microbes or the binding of complement proteins to carbohydrates on the surfaces of microbes. This recognition signal triggers a rapid killing response [16]. The speed of the response is a result of signal amplification that occurs following sequential proteolytic activation of complement molecules, which are also proteases. After complement proteins initially bind to the microbe, they activate their protease activity, which in turn activates other complement proteases, and so on. This produces a catalytic cascade that amplifies the initial signal by controlled positive feedback [17]. The cascade results in the production of peptides that attract immune cells, increase vascular permeability, and opsonize (coat) the surface of a pathogen, marking it for destruction. This deposition of complement can also kill cells directly by disrupting their plasma membrane [12].

Cellular barriers

A scanning electron microscope image of normal circulating human blood. One can see red blood cells, several knobby white blood cells including lymphocytes, a monocyte, a neutrophil, and many small disc-shaped platelets.

Leukocytes (white blood cells) act like independent, single-celled organisms and are the second arm of the innate immune system [18]. The innate leukocytes include the phagocytes (macrophages, neutrophils, and dendritic cells), mast cells, eosinophils, basophils, and natural killer cells. These cells identify and eliminate pathogens, either by attacking larger pathogens through contact or by engulfing and then killing microorganisms. Innate cells are also important mediators in the activation of the adaptive immune system [18].

Phagocytosis is an important feature of cellular innate immunity performed by cells called 'phagocytes' that engulf, or eat, pathogens or particles. Phagocytes generally patrol the body searching for pathogens, but can be called to specific locations by cytokines [16]. Once a pathogen has been engulfed by a phagocyte, it becomes trapped in an intracellular vesicle called a phagosome, which subsequently fuses with another vesicle called a lysosome to form a phagolysosome. The pathogen is killed by the activity of digestive enzymes or following a respiratory burst that releases free radicals into the phagolysosome [19, 20]. Phagocytosis evolved as a means of acquiring nutrients, but this role was extended in phagocytes to include engulfment of pathogens as a defense mechanism [21]. Phagocytosis probably represents the

oldest form of host defense, as phagocytes have been identified in both vertebrate and invertebrate animals [22].

Neutrophils and macrophages are phagocytes that travel throughout the body in pursuit of invading pathogens [23]. Neutrophils are normally found in the bloodstream and are the most abundant type of phagocyte, normally representing 50% to 60% of the total circulating leukocytes [24]. During the acute phase of inflammation, particularly as a result of bacterial infection, neutrophils migrate toward the site of inflammation in a process called chemotaxis, and are usually the first cells to arrive at the scene of infection. Macrophages are versatile cells that reside within tissues and produce a wide array of chemicals including enzymes, complement proteins, and regulatory factors such as interleukin 1 [25]. Macrophages also act as scavengers, ridding the body of worn-out cells and other debris, and as antigen-presenting cells that activate the adaptive immune system [18].

Dendritic cells (DC) are phagocytes in tissues that are in contact with the external environment; therefore, they are located mainly in the skin, nose, lungs, stomach, and intestines (26Guermonprez et al., 2002). They are named for their resemblance to neuronal dendrites, as both have many spine-like projections, but dendritic cells are in no way connected to the nervous system. Dendritic cells serve as a link between the bodily tissues and the innate and adaptive immune systems, as they present antigen to T cells, one of the key cell types of the adaptive immune system (26Guermonprez et al., 2002).

Mast cells reside in connective tissues and mucous membranes, and regulate the inflammatory response. They are most often associated with allergy and anaphylaxis. Basophils and eosinophils are related to neutrophils. They secrete chemical mediators that are involved in defending against parasites and play a role in allergic reactions, such as asthma. Natural killer (NK cells) cells are leukocytes that attack and destroy tumor cells, or cells that have been infected by viruses.

Natural killer cells

Natural killer cells, or NK cells, are a component of the innate immune system which does not directly attack invading microbes. Rather, NK cells destroy compromised host cells, such as tumor cells or virus-infected cells, recognizing such cells by a condition known as "missing self." This term describes cells with low levels of a cell-surface marker called MHC I (major histocompatibility complex) - a situation that can arise in viral infections of host cells. They were named "natural killer" because of the initial notion that they do not require activation in order to kill cells that are "missing self." For many years it was unclear how NK cells recognize tumor cells and infected cells. It is now known that the MHC makeup on the surface of those cells is altered and the NK cells become activated through recognition of "missing self". Normal body cells are not recognized and attacked by NK cells because they express intact self MHC antigens. Those MHC antigens are recognized by killer cell immunoglobulin receptors (KIR) which essentially put the brakes on NK cells [27].

Stylos et al. [28] and Maurer et al. [29], in their publications describing the immune response of sheep against some synthetic polymers of amino-acids having a net negative or neutral charge, noted that upon fractionation of the sera by DEAE-Sephadex chromatography all antibody activity was localized in the y,-immunoglobulin fraction. In contrast to this finding they reported that antibody against DNP conjugated to BSA or DNP conjugated to polymers of aminoa cids was localized in both the V2 and y, immunoglobulin fractions. The study of Zimmering et al. [30] in sheep immunized with testosterone-BSA showed no consistent localization of antitestosterone antibody in yi or V2 immunoglobulin fraction. These investigations and the immune response in the sheep immunized by us could not confirm the observation of Sela and Mozer [31] in the rabbit. From the data presented it seems at least that this mechanism is true in rabbit but not in sheep and other animals [32]. On the other hand, the immune response was analyzed by the named authors in animals highly immunized, meanwhile we have analyzed the sheep immune response weekly. We found that in samples with a high level of anti-hapten antibodies localized in the yV fraction, V2 antibody was not detected or was detected at minimal concentrations. The fact that the three animals give the same immune response permit us to suppose that this is the mechanism in sheep immunized with a strongly immune dominant hapten (DNP) conjugated of HGG. These findings agree with observations by Binaghi [33] in guinea-pigs and by Barth, McLaughlin and Fahey, [34]in mice. In conclusion can be state that use of immunostim has protective effect on sheep's immune response.

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