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IMMUNE RESPONSE MODULATION INDUCED BY MYCOPLASMA AGALACTIAE

Tîrziu E., C. Cumpănăşoiu, M. Sărăndan

USAMVB Timişoara, Facultatea de Medicină Veterinară

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Abstract. The purpose of this research was to assess the effect of the anti-*Mycoplasma agalactiae* vaccine over the specific and non-specific humoral and cellular immune response in sheep. There were also determined the intensity and extent of the immune response, quantified by antibody levels. The results were compared between an experimental lot (vaccinated animals) and a control lot (non vaccinated animals) (4). The effect of the anti-*Mycoplasma agalactiae* vaccine was also assessed by monitoring its influence over the specific and non-specific humoral and cellular immune response, as well as the modulation of the immune response induced by the vaccine in association with an immunomodulator (1, 2).

Our results show an increase of the antibodies titre after the first immunization (primary immune response) which is amplified after the second immunization (secondary immune response) and reaches a maximum level at 45 days. After this point it gradually decreases, to values similar to those registered after the first vaccination, until the end of the experiment (108 days). The probiotic used enhanced the immunogenity of the vaccine, leading to an increased synthesis of specific antibodies, both in the primary and secondary immune response.

INTRODUCTION

Even though *Mycoplasma* induces both a cellular and humoral immune response, the latter is usually the most studied. Thus, the indirect diagnosis is made by using serological tests which reveal specific antibodies present in the infected animal's blood. (3).

The non-specific defence mechanisms includes: complement, properdin, lysozyme, polymorphonuclear cells as well as other various molecular mediators secreted by the cells. *Mycoplasmas* have a wide series of non-specific immunomodulator effects over the cells of the immune system by inducing suppression, by polyclonal stimulation of the B and T lymphocytes, by inducing the synthesis of different cytokines, by amplifying the cytotoxicity of macrophages, NK cells, and T lymphocytes, by stimulating the expression of cellular receptors and by activating the complement system (2).

MATERIALS AND METHODS

The research was made on a group of 30 adult sheep, in mammary pause, divided in two experimental lots: A and B. They were kept in the same conditions and fed the same concentrated fodder. The fodder administered to lot B was supplemented with 1kg/tone of Bio-Mos (Alltech).

In order to stimulate immunity, the sheep were injected with *Mycoplasma agalactiae.* The dose used was 1 ml administered subcutaneous in the flank region, at the beginning of the experiment and after 21 days. The immunomodulator effect of the probiotic was assessed over the specific immune response by antibody levels and over the non-specific immune response

by lysozyme and properdin serum concentration, white blood cell count and the phagocytic index.

RESULTS AND DISCUSSIONS

The Bio-Mos supplemented in the sheep's diet had a strong effect over the humoral immune response. Specific antibody levels measured by indirect ELISA and using a multi-channel spectrophotometer are presented in table 1. The antibody titre, expressed as optical density units, has increased gradually in all animals, both in the control and experimental lot. The highest values were registered in the lot that was administered Bio-Mos (table 1).

The secondary immune response is a lot more intense. It is generated by the renewal of the immunologic memory, consecutive to the second inoculation of the vaccine ("booster effect"). After 14 days from the second inoculation the level of anti-*Mycoplasma agalactiae* antibodies was approximately 7 times higher in the experimental lot than in the control lot (x pr/p = 6,83 ± 1,06). These results are similar with other data we found in literature which claim that the antibodies induced by an inactivated antigen against sheep contagious agalactia, and adjuvanted with natrium hydroxide, are detectable after three months after vaccination (20).

The highest average value for antibody titre (1589±76.72 unities of optical density) was recorded in the experimental lot at 42 days after the second vaccination, consecutive to the secondary antigenic stimulation. Compared to the experimental lot, in the control lot, where no probiotics were administrated, the values were lower: 42 days after the second vaccination, the difference between the optical density units recorded in the control lot and in the experimental lot was of 830,46.

Table 1

The antibody immune response in the control lot

Lot		Time interval between samples					
		Initial	At 21 days	At 42 days	At 63 days	At 84 days	At 105 days
M	x±Sx	230,6± 19,11	279,1± 24,13	490,6± 26,34	759,4± 95,36	329,9± 34,06	255,8± 22,38
	CV.	32,10	33,48	20,79	48,63	39,97	33,88
E	x±Sx	217,1± 29,73	617,7± 61,72	1297,9± 76,52	1349± 62,79	1349,5± 62,79	857,2± 53,51
	CV.	53,03	38,69	15,18	18,64	18,02	24,18

(without Bio-Mos) and experimental lot(with Bio – Mos)

Legend: x = arithmetic mean; Sx = standard deviation; CV = variability coefficient

The highest values for serum lysozyme were recorded in the experimental lot, (vaccinated) and the maximum value of 16,32|ag/cnr was recorded in this lot two weeks after the second vaccination (table 2).

Table 2

The serum lysozyme values in the control (without Bio-Mos) and experimental lots (with Bio-Mos)

Lot		Time interval between samples					
		Initial	At the 21 days	At 42 days	At 63 days	At 84 days	At 105 days
M	X±Sx	12,77± 0,16	13,43± 0,18	14,96± 0,20	14,05± 0,30	12,68± 0,35	10,63± 0,38
	CV.	4,94	5,46	5,33	8,54	10,71	14,15
E	X±Sx	13,28± 0,23	14,64± 0,19	16,32± 0,20	15,90± 0,23	15,37± 0,22	15,14± 0,28
	CV.	6,95	5,15	4,78	5,71	5,78	7,20

Legend: X = arithmetic mean; Sx = standard deviation; CV = variability coefficient

Properdin, the main indicator for the non-specific resistance, had a characteristic evolution during the experimental period in the two lots, (table 3).

At the beginning of the experiment there were no significant differences between properdin levels in the two lots. The average levels were approximately equal but 28 days after vaccination there were found significant differences between the lots, which maintained until the end of the experiment. By analyzing the variations of the serum properdin levels compared to those of serum lysozyme we can see that the properdin level increases until the end of the experimental period. The highest values were recorded 63 days after vaccination (23,46±0,37 mg/100cm^3 ser).

Table 3

The values of serum properdin in the control (without Bio-Mos) and experimental lots (with Bio-Mos)

Lot		Time interval between samples					
		Initial	At 21 days	At 42 days	At 63 days	At 84days	At 105days
M	X ±Sx	16,18 ± 0,21	17,10 ± 0,30	18,78 ± 0,31	20,78 ± 0,55	16,88 ± 0,40	15,80 ± 0,18
	CV.	5,03	6,88	6,51	10,42	9,36	4,55
E	X ±Sx	16,74 ± 0,41	18,24 ± 0,50	21,21 ± 0,43	23,46 ± 0,37	21,29 ± 0,48	21,88 ± 0,43
	CV.	9,55	10,80	7,90	6,19	8,74	7,84

Legend: X = arithmetic mean; Sx = standard deviation; CV = variability coefficient

The results of the haematological tests (white blood cell counts) are presented in table 4. The total number of white cells has increased progressively in both lots, but more evident in the experimental lot that was vaccinated. The highest values were recorded at the end of the experimental period. In the control lot there is no significant increase in the lymphocytes numbers but in the experimental lot, the lymphocyte number has increased both after the first and after the second vaccination. The rapport between the lymphocytes and the neutrophils is reversed.

The phagocytic index has lower values in the control lot during the entire experimental period. The lowest values were recorded at the beginning of the experimental period and at the end of the experiment.

The results found on the phagocytic index are correlated with the results of the blood white cells count. The maximum values recorded in the samples collected the second time, are maintained until the end of the experiment.

Considering the superior results obtained in the lot that was administrated probiotics, in respect of specific antibodies titre, higher lymphocytes numbers and non-specific humoral effectors concentration, we consider that the Bio-Mos probiotics stimulate the general immunological reactivity in sheep.

Table 4

White Blood cells count - leukocyte population configuration
(average levels)

Lot	Sample	Total white blood cells					
			L(%)	N(%)	E(%)	B(%)	M(%)
M	R1	2314,0	49,18	35,34	6,49	0	8,99
	R2	2635,6	51,48	31,18	4,45	0	12,37
	R3	4418,1	52,88	30,86	6,14	0	10,15
	R4	4809,4	56,72	31,43	2,32	0	9,53
	R5	4113,6	52,92	30,35	4,16	0	11,90
	R6	3512,5	47,34	34,04	4,78	0	13,82
E	R1	2743,0	55,42	34,40	4,52	0	5,66
	R2	3525,4	57,41	32,21	6,03	0	4,35
	R3	3705,1	58,82	31,38	4,73	0	5,07
	R4	6135,0	60,45	30,15	4,28	0	5,12
	R5	6012,3	59,88	30,37	4,33	0	5,42
	R6	5813,6	57,53	31,68	5,12	0	5,67

Table 5

Values of the phagocitic index in the control
(without Bio-Mos) and experimental lots (with Bio-Mos)

Lot		Interval of sampling the probes					
		Initial	At 21 days	At 42 day	At 63 days	At 84 days	At 105 days
M	X±Sx	16,86± 1,03	22,26± 1,46	26,86± 1,89	22,66± 1,20	22,33± 1,26	20,60± 1,47
	CV.	23,80	25,54	27,38	20,60	21,97	27,75
E	X±Sx	17,93± 1,16	27,40± 1,61	38,40± 1,35	35,53± 1,51	30,60± 1,55	29,46± 1,62
	CV.	25,24	22,82	13,66	18,03	19,67	21,34

Legend: X = arithmetic mean; Sx = standard deviation; CV = variability coefficient

CONCLUSIONS

Sheep contagious agalactia vaccine induces in sheep a primary and secondary immune response expressed as antibody production that lasts for at least three and a half months.

The antibody titre increases after the first immunization (primary immune response), amplifies after the second immunization (secondary immune response), reaches a maximum level at 45 days and then gradually decreases until the end of the experiment (108 days) to values that are comparable to those following the primary response.

The haematological tests show a progressive increase in the total white blood cell numbers, the maximum values being registered at the sheep in the experimental lot.

The vaccine against the sheep contagious agalactia stimulates the lysozyme and properdin synthesis but the amount is insignificant.

The probiotics potentiate the immunogenity of the vaccine; they determine an increase of specific antibody synthesis, both in primary and secondary immune responses but the differences compared to the lot inoculated only with vaccine is not statistically sustained.

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