STUDY ON THE IMMUNOSTIMULATING ACTIVITY IN CATTLE INDUCED BY POLYSACCHARIDES OF PLANT ORIGIN

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Summary
Immunostimulating activity in dairy heifers elicited by plant polisaccarides orally administered for 20 days was studied *in vitro* by assessing antibody response after vaccination with inactivated or live-attenuated vaccines, leukocytes counting, serum proteins, serum immunoglobulins and blast transformation of peripheral blood lymphocytes.
Results pointed out that the treatment elicited cell-mediated immunostimolation as demonstrated comparating the index of stimulation in Concanavalin A-stimulated lymphocytes detected in treated and non-treated cattle.

Introduction
Within the intensive cattle farming, both dairy and beef farming, numerous are the events that induce depression of the immune system. Many microorganisms are involved in the pathogenesis of disease in cattle, especially respiratory and enteric one. However, only few of them faithfully comply with the well-known Koch's postulates. In fact, most pathogens entirely show their potential when predisposing factors occur, this lead to believe that diseases observed in intensive farming are conditionated-multifactorial. Serious pathological conditions result from the occurrence of stressing factors (transport, over-crowding, conflicts caused by hierarchical balance perturbations in the group, sudden dietary changes) and microclimate (6, 7). In relation to the animal, the said factors can be also marked as endogenous or exogenous. When such events, which can be difficult avoided during the various production cycles occur, a synergistic effect between extramicrobial and microbial factors takes place and this results in the appearance of a clinically manifested disease, the severity of which depends, once again, on a multifactorial complex. This synergistic action expresses in the impairment of organic resistances through the depression of humoral and cell-mediated immune function (2, 11).
Many diseases find in vaccination the rational response that may contain the action of specific pathogens. However, vaccination is restricted to the valence provided by the vaccine, to the possible interference given by passive antibodies of maternal origin, as well as to the occurrence of immunosuppressive states evoked by the factors mentioned above.
In consideration of this, the use of immunostimulating or immunomodulating aids that can be used during the critical phases of the productive cycle arouses a growing interest . The pharmacological intervention directed to the stimulation of the cell complex which reacts not specifically in the "first line" of defense is called para-immunization (3). Substances that can evoke immunomodulation or para-immunization have been jointly defined as immunomodulators or inducers of para-immunity.
As regards the classification of these aids, it is characterized by their origin rather than their site of action. Therefore we talk about biological (bacteria and their products, yeast extracts, viruses, parapox in particular, regulator physiological products) and chemical immunomodulators (levamisole, isoprinosine, avridina, azimexone, synthetic polyribonucleotides) (9). In the context of biological immunomodulators there are substances of vegetable origin, and in particular polysaccharides from different plants.

The purpose of this study is to evaluate the immunostimulatory activity in cattle of a product based on polysaccharides fractions of vegetable origin.

Materials and methods.

Design of experiment

The substance object of the study is made up of a commercial product based on in equal parts mixed polysaccharides obtained from herbs belonging to different species (*Echinacea purpurea, E. pallida* and *E. angustofolia*) of *Astracea* family. The treatment provided the administration of 100 grams/head/day of the product for 20 days.

Overall n. 60 belonging to the same farm Friesian cows between 12 and 18 months aged and not subjected to BHV-1 prophylactic immunization were considered.

During the observation period, no specific or non-specific immunizing interventions in addition to what is following described have been performed.

The animals have been divided according to the following intervention protocol:

Group A: 10 animals, untreated, BHV-1 monovalent inactivated vaccine administered;
Group B: 10 animals, untreated, BHV-1 monovalent live-attenuated vaccine administered;
Group C: 10 animals, untreated, not vaccinated;
Group D: 10 animals treated, BHV-1 monovalent inactivated vaccine administered;
Group E: 10 animals, treated, BHV-1 monovalent live-attenuated vaccine administered;
Group F: 10 animals, treated, not vaccinated.

In all cases, vaccination was performed within 10 days from the end of the treatment object of the study.

Therefore, the serum antibody titer against BHV-1 was individually evaluated.

On serum, whole blood and haematic cell fraction collected within 10 days after the end of treatment (or at the moment of vaccination) (1st sample) and after 20 days (2nd sample) were detected:

a) antibody titer against BHV-1, by means of serum neutralization (SN) (5);
b) white blood cell counts (cells/ml x 10^3), using an automatic device (*Medonic*);
c) total serum protein (g/100 ml) (8);
d) serum gamma globulins (mg/ml), calculated in relation to the percentage of the expressed fraction that results from the electrophoretic migration of the serum on cellulose acetate and densitometric reading of protein bands;
e) lymphocyte stimulation through a 3H-thymidine incorporation assay after stimulation with Concanavalin A and evaluation of the stimulation index (SI: expressed as c.p.m. ratio between the average value of incorporation of 3H-TdR of stimulated cells and the one of not stimulated cells) (10).

For each considered assessment, the arithmetic mean and the standard deviation within the indicated individual groups was calculated. In addition the means of each group were compared by means of the *Student's t* calculus.

Results

The administration of the product did not cause the onset of clinically detectable changes in animals subjected to the treatment. Likewise, their general state and their large organic functions

did not undergo significant changes.
The results related to laboratory investigations that were aimed at the determination of immune serum-blood parameters referred to above are indicated in Table 1.

Table 1. Distribution of immune serum blood parameters (arithmetic mean ± standard deviation) in the different groups of cattle.

Group	Sample	BHV-1 SN Titer	Leucocytes	Total sieroproteins	Gamma Globulins	Lymphocytic sensitivity Index
A	1° 2°	0,8±1,1 10,4±5,4	8940±1850 9741±768	6,8±0,7 6,5±1,2	23,8±3,5 25,1±5,2	12162±1722 11120±2140
B	1° 2°	1,0±1,1 9,0±5,1	8140±751 8450±831	7,1±1,8 6,9±2,4	24,2±8,3 25,1±6,2	11910±2411 12022±4120
C	1° 2°	1,2±1,4 1,4±1,6	9430±1830 9650±2110	6,8±3,1 7,4±3,2	22,9±4,3 21,9±3,6	11908±4110 12022±3115
D	1° 2°	1,0±1,4 8,0±4,6	9120±1750 8980±2420	6,4±1,2 6,9±1,2	24,2±6,2 23,8±3,2	14995±2284 15022±3140
E	1° 2°	0,9±0,5 8,4±3,5	8510±2420 9118±2511	7,2±2,2 6,7±1,9	25,1±6,2 24,8±5,2	15611±3140 12221±2120
F	1° 2°	0,8±1,1 0,8±0,8	8740±1150 9005±2415	6,9±2,3 6,7±1,7	23,6±3,2 24,8±5,6	15882±1210 12034±1898

With regard to the statistical evaluation, by the *Student's t* calculus, and directed towards the comparison of data obtained in the different groups of animals, the comparisons between the following groups resulted statistically significant ($P < 0.01$) (below only the comparisons that can be considered useful for the interpretation of the obtained results are showed):

BHV-1 SN Titer:
A1° vs A2°, B1° vs B2°, D1° vs D2°, E1° vs E2°,
A2° vs C2°, B2° vs C2°, D2° vs F2°, E2° vs F2°;

Lymphocytic stimulation index:
D1° vs A1°, D1° vs B1°, D1° vs C1°,
E1° vs A1°, E1° vs B1°, E1° vs C1°, E1° vs E2°,
D2° vs A2°, D2° vs B2°, D2° vs C2°, D2° vs E2°, D2° vs F2°,
F1° vs A1°, F1° vs B1°, F1° vs C1°.

Discussion and conclusions

In order to reach a correct critical evaluation of the obtained results we consider appropriate to make some preliminary remarks. We make specific reference to the fact that the treatment object of the study interested cattle which did not show clinical states related to immunocompromise. It follows that any benefit arising from the treatment of immunocompromised animals can not find an objective evidence in the performed study. As regards, here we would like to remark that pathological manifestations observed in the intensive cattle farming, both dairy cattle and beef cattle farming, more often appeal to states of immunodepression. Such conditions find their

genesis in elements of infectious and also managerial nature. However, it should be also observed that the diagnostic evidence of them can not be based on clinical elements but must find an objective instrumental feedback through appropriate laboratory investigations in order to assess the related to the immune functions complex of the individual parameters and subsequent projection within the farm.

Having said this, the statistical evaluation of the data that we have acquired would indicate a non-specific immunostimulatory activity of the treatment, particularly addressed to the cell-mediated immunity. In fact, the results related to the lymphocytic sensitivity indices after a non-specific stimulation indicate a significant difference in the treated groups, compared to the untreated ones. This effect seems, however, to run out within 20 days after the end of treatment.

In contrast, with regard to other considered parameters no related to the treatment significant changes have been detected, in particular those related to the average serological titres obtained after vaccination to BHV-1 with an inactivated vaccine and with a live attenuated vaccine.

With this regard, however, it must be observed that in the course of the 2nd blood sample (performed after 20 days from the end of treatment), the animals subjected to a vaccination treatment with an inactivated vaccine (D2° group) maintain an average lymphocytic sensitivity index higher than untreated animals (A2° groups, B2° and C2°) but also higher than those that have been vaccinated with a live-attenuated vaccine (group E2 °) and also higher than those treated but not vaccinated (group F2 °).

In support of this, the non-specific immunostimulatory activity referable to the adjuvant component only present in the inactivated vaccine can be considered. Such action would reinforce a non-specific cell-mediated immunity induced by the treatment, which in absence of the vaccination would tend to run out.

As already indicated in the literature (1, 4), in conclusion we consider that the administration of products with an immunostimulatory activity may represent a possible pharmacological support able to contrast the onset of pathological states, in particular through the stimulation of the first line organic defence against microbial attackers. However, this should not be considered a substitute instrument but only an adjuvant of the use of specific immunization interventions.

Thus, the use of aids of plant origin, provided with high biocompatibility and with the possibility of oral administration through the diet, requires adequate attention. In this regard, we however consider essential to proceed with the preliminary validation of the different products and related intervention protocols through a correct experimental support.

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