

VEGETAL EXTRACTION INDUCED INNATE CELL-MEDIATED IMMUNE RESPONSE IN VACCINATED HENS

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Summary

The need for non-toxic, novel adjuvants with increased bio-availability and efficacy, added to an improved accessibility represent a primary aim in modern veterinary vaccinology. The experiment was carried out on two groups of birds, vaccinated (n=25) and saline injected control (n=35). The vaccines used were directed against Newcastle disease, infectious bursitis, infectious bronchitis and viral arteritis (Quadractin) as well as against fowl plague.

In vitro carbon particle clearance test was used to monitor the phagocytosis before and three weeks after the vaccination. Spectrophotometrical readings of the optical densities of a blood and carbon particle mixture, with alcohol and alcoholic nettle extraction, respectively, or without supplements, were done after 20 and 30 minutes of incubation at 37°C. The alcoholic nettle plant extraction sharply enhanced the phagocytic activity when compared to the control, non-vaccinated birds and the *in vitro* control variants. The results of the research led to the conclusion that, on short term, the alcoholic nettle plant extraction sharply enhances the *in vitro* phagocytic activity, suggesting a possible use in stimulating the innate cell-mediated immune response during priming and therefore a better encounter of the vaccine antigen.

The investigations aiming to establish the adjuvant qualities of vegetal extraction in animals are extremely few and relatively recent (Kong et al., 2004; Kotrbacek et al., 1994). Thus, effects of various Chinese medicinal plants were monitored in comparison with classical adjuvants (Freund's complete adjuvant), using the Newcastle virus as an antigen to prime the birds (Wong et al., 2005; Brewer J.M. et al., 1996). The vegetal extraction mixture augmented the *in vitro* lymphocyte proliferation as well as antibody synthesis, being capable to intervene on mechanisms involved with the adaptive immunity (Castro et al., 1993; Morein et al., 1996).

The need for non-toxic, novel adjuvants with increased bio-availability and efficacy, added to an improved accessibility represent a primary aim in modern veterinary vaccinology (East et al., 1993). Medicinal plants are, under these circumstances, an easily accessible source, with partially proven therapeutical (Bror et al., 1996; Burdin et al., 2004).

Experiments regarding the effects of certain vegetal extractions on the immune parameters represent a priority for the last decades' research (Stosskopf, 1984). The use of vegetal extractions in classical therapeutic protocols could

favorably influence the non-specific defense capacity of the organism, by the complex immunological effect exerted (Spînu et al., 1998).

Testing the *in vitro* functional potential of the phagocytes provides the information on their capacity to recognize and subsequently engulf "non-self" particles.

The purpose of this study was to investigate the manner alcoholic nettle (*Urtica dioica*) extractions influence the phagocytosis under a single antigenic priming, in adult hens.

Materials and methods

The experiment was carried out on two groups of birds, vaccinated (n=25) and saline injected control (n=35), on a laying hen farm of 1,300 individuals. Blood was sampled on sterile heparine (50 UI/ml) by puncturing the wing vein. Two samplings were performed, on the day of the primary antigenic stimulation and three weeks later. Two inactivated, water-in-oil emulsified vaccines, against Newcastle disease, infectious bursitis, infectious bronchitis and viral arteritis (Quadractin) as well as against *Pasteurella multocida*, serotypes 1,3 and 5 (Cholerin triple), were administered subcutaneously, in a dose of 0,5 ml/bird.

At each sampling, the blood samples were subjected to the carbon particle inclusion test (Ghegariu et al., 2000). Three variants were performed for each blood sample: control (I), where only 1.5 μ l of the carbon particle suspension were added, alcohol treated (II), where the blood sample was supplemented with the same amount of carbon particle suspension and 6 μ l of alcohol, and *Urtica dioica* extraction treated (III), where the alcohol was replaced by the alcoholic vegetal extraction. For each variant, 0.50 ml portions of heparinized blood were mixed with the supernatant of India ink, which was obtained by centrifugation at 6000 rpm for 40 min (Karl Zeiss ultracentrifuge). 0.15 ml of each mixture were transferred immediately to 2 ml of saline and the remaining of each mixture was incubated at 37°C. After 20 minutes, another 0.15 ml of the samples were transferred to saline and the incubation was continued up to 30 minutes, repeating the operation.

All tubes containing saline, and the samples were centrifuged at 800 rpm and the supernatants were read spectrophotometrically ($\lambda=535$ nm, $d=0.5$ cm). There was a decrease in absorbance with time as carbon was phagocytized.

The phagocytic index was calculated as optical densities of the supernatants at the two times of incubation for each group of birds. Mean values and standard deviations were calculated and the statistical significance of the values was considered.

Results and discussion

Current trends in medicine tend to include natural products in therapy, without mixing allopathic and homeopathic treatments. Natural products gain more

and more in comparison with chemically obtained ones. Within this framework, researches on the immune stimulating activities of vegetal extractions were successful, with obvious immune modulating effects. Briefly, the use of medicinal plants and their extractions as adjuvant for therapy, although known for centuries, is far from being an closed subject. Medicine and biochemistry show a continuously increasing interest to this field, resulting in the introduction in practice of novel preparations, gaining new meanings within modern prophylactic and therapeutical alternatives (Allison and Byars, 1986; Audibert and Lise, 1993; Vogel, 2000).

The phagocytic activity for all the *in vitro* variants (control, alcohol and nettle extraction treated) in the two groups of birds were presented in tables 1, 2 and 3.

Table 1

Spontaneous phagocytic activity

	Sampling I			Sampling II - M			Sampling II - V		
	0	20	30	0	20	30	0	20	30
Mean	0.139	0.034	0.026	0.156	0.032	0.033	0.158	0.030	0.025
Stdev	0.055	0.019	0.015	0.047	0.010	0.009	0.031	0.014	0.013

M- Control group; V-vaccinated group of birds

Monitoring the spontaneous variant (control), it could be seen that before the antigen priming, it sharply decreased during the first period and much less during the second period of incubation. After the vaccination, the behaviour for the spontaneous phagocytic activity was similar, but there was no decrease seen during the second incubation period. In the vaccinated group, the spontaneous phagocytosis had a similar dynamics to that of the pre-priming moment. The differences between the two samplings for spontaneous phagocytosis were statistically non-significant.

Table 2

Alcohol induced phagocytic activity

	Sampling I.			Sampling - M			Sampling - V		
	0	20	30	0	20	30	0	20	30
Mean	0.141	0.090	0.026	0.224	0.063	0.041	0.247	0.053	0.034
Stdev	0.035	0.014	0.005	0.032	0.026	0.016	0.025	0.028	0.016

M- Control group; V-vaccinated group of birds

The *in vitro* alcoholic treatment decreased significantly ($p < 0.05$) the phagocytosis before the antigen priming, but increased it after the priming especially in the vaccinated group, where the descendent slope was sharper. Alcohol, in the used doses seemed to enhance the phagocytosis, an effect mentioned for alcoholic extractions of other plants, such as *Echinacea angustifolia* (Percival, 2000).

While for the first moment of testing, the nettle plant did not induce spectacular changes of the phagocytosis, it managed to significantly increase the phagocytosis of the control group by the end of the *in vitro* testing period. Still, it induced a severe hemolysis, that interfered the reading, by the end of the reading period in the vaccinated group.

Monitoring the very fast dynamics of the phagocytosis during the first 20 minutes of activity, one could presume that an *in vivo* therapy with the nettle extraction would enhance phagocytosis, with a possible limiting effect of the hemolysis. This effect might also be a read reduction of phagocytosis through the antiinflammatory effects of the nettle plant, that might discontinue the the activity of various cytokines and prostaglandins produced by the circulating cells (Espinosa and Bermudez-Rattoni, 2001).

Table 3

Alcoholic nettle extraction induced phagocytic activity

	Sampling I.			Sampling - M			Sampling - V		
	0	20	30	0	20	30	0	20	30
Mean	0.135	0.028	0.025	0.252	0.054	0.025	0.265	0.034	0.179
Stdev	0.028	0.006	0.009	0.050	0.026	0.011	0.074	0.019	0.070

M- control group; V-vaccinated group of birds

Conclusions

The results of the research led to the conclusion that, on short term, the alcoholic nettle plant extraction sharply enhances the *in vitro* phagocytic activity, suggesting a possible use in stimulating the innate cell-mediated immune response during priming and therefore a better encounter of the vaccine antigen.

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